Milk and Dairy Products in Human Nutrition

Milk and Dairy Products in Human Nutrition

Production, Composition and Health

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Preface

Milk is known as nature's most complete food, and dairy products are considered the most nutritious foods. On the other hand, the traditional view of the role of milk has been greatly expanded in recent years beyond the horizon of nutritional subsistence of infants. Milk is now recognized as more than a source of nutrients to mammalian neonates and for healthy growth of children and nourishment of adult humans. Milk contains biologically active compounds besides its major proteins, casein and whey proteins, that have important physiological and biochemical functions with significant impact on human metabolism, nutrition and health. Numerous milk-borne biologically active compounds have been proven to have beneficial effects on human nutrition and health, including antimicrobial, biostatic, antihypertensive, angiotensin-converting enzyme (ACE)-inhibitory, antiadhesion, antidiabetic, anticholesterol, anticarcinogenic, immunomodulatory, anticariogenic, antiobesity, probiotic, and prebiotic activities. Examples of these compounds include β-lactoglobulin, α-lactalbumin, lactoferrin, immunoglobulins, lysozyme, lactoperoxidase, peptides from caseins and whey proteins, glycomacropeptides, phosphopeptides, oligosaccharides, conjugated linoleic acid, polar lipids, gangliosides, sphingolipids, medium- and short-chain fatty acids, monounsaturated and polyunsaturated fatty acids, triglycerides, milk minerals, growth factors, hormones, vitamins, and nucleotides. Among the many valuable constituents in milk, the high level of calcium plays a particularly important role in the development, strength and density of bones for children and in the prevention of osteoporosis in older people. In addition, calcium has also been shown to be beneficial in reducing cholesterol absorption, and in controlling body weight and blood pressure.

No dairy-related book published so far has ever comprehensively covered the whole spectrum of milk secretion, production, sanitary procedures, flavor, chemistry, processing technology, nutritional and health properties of milk and manufactured products from a variety of different dairy species in relation to human nutrition and health. We anticipate that this book will benefit readers around the world, including students, scientists, and especially health-conscious consumers who are looking for scientific information on production systems, mammary secretion, milking procedures, quality standards, sanitary procedures, milk allergy, lactose intolerance, bioactive compounds, therapeutic substances, and sensory and flavor components in milk and manufactured dairy products from different dairy species as well as human milk.

Because of the unavailability of cow milk and the low consumption of meat, the milks of non-bovine species such as goat, buffalo, zebu, mithun, sheep, mare, yak, camel and reindeer are important daily sources of protein, phosphate and calcium for people in developing or under-developed countries, where non-bovine dairy species play an immensely important role in the supply of food and nutritional subsistence. Uniquely, this book covers the products of all the different dairy species currently consumed by humans, and which have significant impact on human well-being and survival. This work will be an important and comprehensive reference book, and is intended to deliver the best available knowledge and up-to-date information by world authorities and experts in dairy science and technology. From a roster of 108 invited scientists, we have assembled a group of internationally reputed expert scientists in the forefront of milk and dairy products, food science and technology in producing this extensive scientific work.

A diverse audience may be expected for this book. We anticipate that it will become a textbook or reference book for classroom situations (dairy science, food science and technology-related courses) at colleges/universities, libraries, and governmental agencies. The main readership is likely to include students around the world majoring in dairy and food sciences and nutrition, and professionals such as food scientists, food technologists, dairy manufacturers, nutritionists, medical and health professionals, and health-conscious consumers. It is hoped that more inclusive audiences would include the dairy industry, milk producers, dairy processors, dairy marketers (retail and wholesale), food industry writers and magazine publishers, veterinarians, libraries (public and technical), food sanitarians and regulators and administrators, agricultural

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schools and colleges, consumers, and connoisseurs. More importantly, we believe that the most significant audience for this book should be the most important end-user, the general consumer, health-food lovers, allergy specialists, infant formula specialists, and other dairy species enthusiasts. The contents of this book are unique and will be an especially important resource for those people seeking nutritional, health and therapeutic or product technology information on milk and dairy products from species other than the dairy cow.

Only a few books and a number of journal papers on milk and dairy products have been published in relation to human nutrition and health. However, all the books contain fragmented information or narrowly focus on milk and dairy products, and none of them have covered the whole spectrum of milk production. We hope that this unique, in-depth, specialized and extensive depository of information and its comprehensive coverage of the scientific literature will have a lasting impact on the understanding of milk and dairy products in human nutrition and health.

The focus of this book is to call attention to the global aspects of the dairy industry and is especially timely as a new report from the Food and Agricultural Organization of the United Nations (*Cheese Market News*, vol. 32, no. 31, p. 8) indicates that global milk production has increased by 2% during the last 10 years and will continue to increase at this rate in the next 10 years. However, 70% of this increase will occur in the developing countries, with India and China accounting for nearly 40% of the projected increase, and by 2013 the developing countries will surpass the milk production level in the developed countries. Whole milk powder is expected to become the fastest growing dairy product, followed by fermented products. Per-capita consumption of milk in Europe and North America is twice that in other countries, but these other countries are expected to narrow the gap by as much as 22%. The global export of dairy products is also expected to grow tremendously, again indicating how timely the appearance of this book is.

This book also shows in great detail where and how progress in milk production of other species is possible in order to better satisfy growing human demands. The dairy cow is the worldwide model in achieving high production levels through genetic selection, with emphasis on excellent udder and teat formation, long lactations, and efficient and sanitary mechanical milking procedures without the need to have a calf present for milk let-down. In conclusion, we hope this book will be widely accepted around the world.

It has proved to be quite challenging to produce this book with a decidedly international scope, to find outstanding scientists outside the Western world who would agree to write from their experience about the specified topics and in English, not their native language, albeit with considerable assistance from the book editors and the editorial staff at the publishers, Wiley-Blackwell. Very special thanks are expressed to the publisher, Mr. David McDade, for giving the editors the opportunity of publishing this comprehensive work in the field, and also all individuals at Wiley-Blackwell who have made significant contributions in bringing this project to fruition. Our appreciation is extended to the freelance project manager, Ms. Alison Nick, as well as the teams involved in the editing and typesetting of this publication, for their outstanding work and flexible understanding of the real challenges in the writing of the 31 chapters of this book to achieve logical language, continuity and clear presentation of tables and figures. Finally, we are deeply indebted to Mrs. Eun Young Park and Mrs. Lizzy Haenlein for their strong and unwavering support, commitment and sacrifice in the journey of the completion of this work.

Y.W. Park and G.F.W. Haenlein
Editors

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1
Production Systems around the World

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1.1 ECOLOGICAL CONDITIONS

One can distinguish between temperate, subtropical, tropical dry, tropical humid and montane conditions, each offering different possibilities for milk production, and which are the basis for different production systems (Seré & Steinfeld, 1996).

The chief dairy zones are the lowlands of the temperate climatic zone (Table 1.1). Often these receive high rainfall, which is unfavourable for cropping and the land is best used as grassland. Less than 0.5 ha may carry an animal unit (AU). Similarly, land on high-altitude mountains, for example the Alps and Pyrenees in Europe, at 1500–2000 m above sea level, is not useful for cropping because of high precipitation and short vegetation period but is used as a welcome addition to grazing by dairy animals from valley farms with limited cultivable land.

The tropical environment is generally less suitable for high-producing European dairy animals, mainly because at elevated ambient temperatures the animal needs to expend energy for dissipating excess heat. Metabolic heat production is reduced by reducing feed intake and lowering metabolic rate, and this is not compatible with high milk production (Rhoads *et al*., 2009). As heat dissipation is mainly by water evaporation, high air humidity further aggravates the negative effects of the tropical environment. In addition, the humid tropics are not suitable for high-producing dairy animals because night temperatures mostly remain above 30°C and the metabolic heat cannot be dissipated (Preston & Leng, 1987).

Cattle of the *Bos taurus* genus are of little importance in the equatorial zone with extreme rainfall. Although vegetation may be abundant, with fast growth and early maturity, the plants have a high fibre content and consequently are difficult to digest and their nutrient value is low. Although increased use of the Amazonian basin for cattle-keeping demonstrates that a feed base can be created there, the preceding deforestation is not acceptable for ecological and socioeconomic reasons (Butler, 2011).

In tropical dry-lands, lack of forage due to insufficient rainfall is the limiting factor, in addition to elevated temperature. More than 400 mm rainfall is generally required to sustain cattle. In the humid savannah with 500–1000 mm rainfall, between 4 and 10 ha may be required to carry 1 AU, depending on the annual rainfall pattern. With higher and less variable rainfall only 2 ha may be required for 1 AU and only 0.5 ha on improved pasture. However, where rainfall is sufficient and feed supply is good, cattle-keeping competes with cropping for surface, capital and labour. Although average annual rainfall is not sufficient to determine the suitability of an area (because the availability of water for plant growth depends on the annual distribution pattern and the evaporation of water), it is a useful approximation. Where rainfall is extremely low and erratic, with regularly occurring extended drought periods, the feed base is insufficient to meet the nutrient requirements of cattle and more than 50 ha may be required to carry one tropical AU (De Leeuw & Tothill, 1990). Although conditions are less suitable in semi-arid and sub-humid

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Table 1.1. Pasture area required to sustain livestock by ecological zones in the tropics.

Ecological zone	ha/tropical AU*	Comment
Temperate lowlands	0.5	Grassland more suitable than cropping
Tropical highlands	0.5	Competition with intensive cropping
Tropical, humid	0.5–2	With improved pasture
Humid savannah	4–10	
Subtropical, dry	>50	

*Tropical AU = 250 kg liveweight.

Source: based on data from De Leeuw & Tothill (1990).

tropical areas, much milk is produced here because of the preponderance of small-scale farmers who depend on it. In sub-humid Africa, milk production may be hampered by disease (e.g., trypanosomiasis) but disease-tolerant breeds can be kept even for milk production (Agyemang, 2005).

Tropical highlands with temperate climatic conditions and sufficient rainfall may be ideal for cattle-keeping. Here, less than 0.5 ha may be required per AU and dairying is possible even with temperate breeds, although with a high density of human population and high soil fertility competition from cropping may leave little room for livestock, unless both operations are integrated.

1.2 SYSTEMS

1.2.1 Small-scale milk production

Very early in history, people must have learnt to milk. Certainly boys herding the flock tasted some milk directly from the udder and milk was extracted from the udder of animals which had lost their young. Later on, this will have developed systematically, for example by early slaughter of excess male progeny. Eventually, rearing of youngstock was combined with milking whatever quantity was possible without compromising the development of the young. The so-called dual-purpose system, where milk production is combined with rearing and even fattening of all male progeny, was the prevailing system in small-scale farming over the centuries and is still prevalent today in those areas where small farms dominate (Falvey & Chantalakhana, 1999).

In the past it was difficult to generate an adequate family income with agricultural activities alone on small farms (as prevails in many European countries) with limited production resources (land and capital) but possibly excess labour. Labour-intensive livestock keeping, dairy animals in particular, provided the possibility to generate additional income. Thus, dairying based mainly on pasture supplemented with agricultural by-products, was part of an integrated agricultural smallholder family enterprise in most countries. It is estimated that around 14% of the world's population depend directly on dairy production for their livelihoods. In order to support smallholders in Europe with a view to the socioeconomic impact (on average, dairying accounts for about 20% of agricultural output in EU countries), milk production was heavily subsidised by market intervention (price support and milk quotas, see section 1.13). Similarly, in the USA the milk price was stabilised by subsidies, in Canada by milk quotas.

Even today, in the tropics and subtropics under rain-fed conditions, families living on a hectare or two cannot survive economically with crops alone. Livestock production on these farms, in addition to improving family nutrition, provides a higher return on farmers' labour and land. Milk production allows cash to be earned daily, even with little equipment and inputs, for example a single dairy cow or some goats or a Zebu cow. Livestock also add security to the family enterprise. Even landless peasants may benefit from this opportunity. Furthermore, it is a source of organic material and soil nutrients generally lacking in such systems. Small-scale milk production of this nature can be successful with local resources (breeds, feeds, management). Women's smallholder dairy development in East Africa illustrates the promise that a new livestock activity can offer to a farming system under economic stress (Owango *et al*., 1998). Whenever conditions are improving and milk production for the market is the aim, better-responding genotypes are required that contribute earlier maturity, better reproductive function during lactation, and better milkability. While some within-breed improvement through selection works well, this is a long-term effort and its sustainability under the prevailing conditions in developing countries is rarely ensured. In particular, the necessary programmes to maintain pure Zebu (*Bos indicus*) breeds and strains is critical for their survival, while imported European *Bos taurus* breeds are more attractive for crossbreeding for milk yield improvement.

Specialised milk production is economical only if about 3500 kg milk can be sold per cow yearly. In the tropics, this performance is generally not attained with forage alone. Also, milk replacers for calf rearing are generally not available (see section 1.9). Therefore, production systems with limited milk production (approximately 1500 kg of sold milk in 300 days) combined with rearing a calf per year by the cow (with forage and limited feed supplements) are preferred over specialised dairy and meat production (Preston & Leng, 1987).

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The extent of the contribution to overall milk production by local and *Bos indicus* breeds is difficult to assess as breeds are not considered in dairy statistics of different countries. It used to be very high in Central and South American, Asian and African countries in the past, and it still will be in subsistence production systems. However, with increasing intensification and crossbreeding the contribution of local and pure Zebu breeds is diminishing.

1.2.2 Specialised milk production in large commercial dairies

During the 1950s and 1960s in industrialised countries, farms increasingly specialised. Farms with multiple activities tended to give up dairying as a sideline, while those continuing were becoming larger and taking advantage of economies of scale. Where optimal use of limited agricultural resources does not have to be considered, during the twentieth century dairying has developed into large specialised operations with highly productive dairy breeds, advanced technology and capital-intensive systems of production. Examples of technological innovations widely adopted by dairy farmers include (Laister *et al*., 1999; USDA, 2009):

- indoors feeding with high inputs and sophisticated feeding systems;
- elaborate animal housing;
- careful computer-assisted herd management including feeding, reproduction and health;
- modern, largely automated milking equipment in efficient milking parlours;
- on-farm refrigerated bulk milk tanks;
- mechanised waste-handling systems.

In these systems, *Bos taurus* cows may be milking up to 20 000 kg per lactation period of 305 days. Although investment in buildings and facilities, cost of feed procurement and herd management may be quite high, profitability is ensured by high production efficiency. As feed conversion is more efficient with milk production than with fattening, these farms do not consider rearing excess calves not needed for herd replacements, and they dispose of them as early as possible. Large dairy operations were established in some socialist countries: Russia, Poland, Bulgaria, Syria, Nicaragua, Cuba. Typically they comprised several units of about 100–500 milking cows each, with separate barns for calves, heifers, dry and milking cows, and milking parlours sometimes used around the clock. Other features included total mixed rations based on maize silage and alfalfa hay prepared and distributed with mobile mechanical feeders (Lammers *et al*., 2000). Similar operations can be found even in developing countries, where they supply the affluent market of the capital cities.

A minimum viable herd size is nowadays considered to be about 100 milking cows (*Bos taurus*). For instance, in the USA between 1997 and 2006 the proportion of herds with less than 100 cows decreased from 41% to 21%, whereas the proportion with more than 500 cows increased from 24 to 47% (USDA, 2008), and two-thirds of all milk was produced on farms with more than 100 cows in 2000 (Blayney, 2002). Some operations are huge, comprising several thousand cows. In 1998, the top 20 US dairies were ranked by *Successful Farming Magazine* (Looker, 1998). The smallest of these farms had 6500 cows and the largest 18 500. The ever-increasing number of large commercial enterprises benefit from economies of scale, but raise socioeconomic concerns because they are not only crowding out small farmers, but also the agrarian and rural structure is changing (i.e. the disappearance of ancillary activities such as milk collection and artisan processing).

Recently, interest is growing in organic (biological, ecological) dairying. Regulations for official recognition differ between countries but typically stipulate the following in Europe (Borell & Sørensen, 2004; European Union, 2007b):

- half of the total feed intake, both grazing and barn feeding, must originate from the farm;
- no mineral fertilisers or pesticides may be used;
- the time period between drug administration and milking must be twice that of conventional production;
- parturient cows shall be in individual loose boxes;
- calves must receive non-processed natural milk for the first 10 weeks.

Although these practices and the produce appeal to consumers, there may be little advantage with regard to welfare, health and reproduction of the cows (Fall & Emanuelson, 2009; Langford *et al*., 2009). The number of organic dairy farms is still limited, for example less than 2% of all dairies in the USA (USDA, 2007). The cost of producing organic milk compared with conventional production was higher in 2005, but was compensated by higher sales price in most farms investigated in the USA (McBride & Greene, 2007).

1.2.3 Dairy ranching

In dairy ranching in the tropics, cows are kept mainly for raising young feeder stock. If their milk-producing potential exceeds the calf's requirement, some milk may be extracted in a dual-purpose system. Local breeds or breeds crossed with imported 'exotic' European cattle are kept. Cattle possessing a high genetic content of Zebu are preferred as dual-purpose breeds. They are adapted to the environment, but they can be milked only in the presence of their calves. The cows are separated from their

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calves in the evening, milked in the morning and spend the day with their calves. In the evening there is no milking. The lactation period is short. The proportion of cows milked in a herd changes but is generally low. Milk production varies over a wide range depending on time of the year and feed supply. Accordingly, the contribution to farm income of revenues from the sale of milk and from slaughter animals varies.

1.2.4 Urban dairies

Urban dairies (the term 'dairy' is used for both dairy herds and milk processing or creamery factories) are situated in the outskirts or even in the centre of major cities. They were originally established by traders in order to meet the demand for clean fresh milk and to avoid the risk of adulteration in the intermediate trade. The risk of disease transmission was not considered because customarily milk was boiled before use. The system was widespread in all major cities until the early twentieth century. Today it is still frequently found in India and Pakistan but more often with buffaloes and Zebu crosses rather than with pure European cattle. Feed is provided from owned farmland or common land, or even purchased (Dost, 2003). City dairies buy cows after parturition and milk them over the course of one lactation. Calves are used only for stimulating milk let-down and often virtually starve to death. Cows are not bred during lactation. When dry, they are sold off either for slaughter or for re-breeding; replacements are purchased from breeders. Today, for sanitary reasons these dairies are banned from cities in many regions. In India for example, city dairies, which were quite common in most major cities, have been banned and located out of urban areas (deWit *et al*., 1996). They were replaced with varying success by government-sponsored milk colonies or other efficient dairy enterprises.

1.2.5 Pastoralists

The term 'pastoralist' (from Latin *pastor*, herder) refers to livestock keepers who live entirely with and from their animals (FAO, 2001). Typically they practise non-sedentary systems, either nomadism or transhumance. The pasture (common or public grazing ground) that can be grazed by a herd is limited by the distance the animals are able to travel daily between night enclosure and watering points. The number of animals a family can keep is limited by the feed available in this area and by the available labour force. When this herd is not sufficient to sustain a family all year round, non-stationary systems of livestock production have developed. In these, grazing grounds are changed at more or less regular intervals. Pastoralism is of major importance in sub-Saharan and North Africa, Mongolia and Siberia. It exists to a minor extent in western, central–east and south Asia, and in Latin America. It is estimated that worldwide 40 million people are pastoralists (Harris, 2000). Other estimates are much higher, for example 20 million households on 25% of the world's land surface (Degen, 2007). However, pastoralists are increasingly under pressure by politics and agriculturists and true nomadic systems are becoming rare in many countries.

In transhumance systems, families settle in permanent dwellings but move their herds seasonally between dry and wet, summer and winter, plains and mountain pastures, riverine zones flooded during the rains but offering plenty of feed in the dry season. Grazing grounds may be changed several times during the year; some may be used for only very short periods. Herds are accompanied by a few members of the family whereas the core family remains at home (Niamir, 1990). Transhumance is practised in the Mediterranean basin, in the Alps, Pyrenees, Balkan countries, in western and central Asia, in Africa and Latin America (Blench, 2001) but is limited by country border controls.

In contrast, nomads do not have permanent houses and the whole family moves with their huts and tents following the herds, changing locations to where forage and drinking water are available. The rhythm of their movements is determined by the rainfall pattern and season and the availability of feed and water. Routes may vary between years but movements are not erratic but rather follow certain patterns. However, deviation from standard routes can be frequent and is caused mainly by the erratic nature of rainfall in dry zones but also by security considerations (civil strife). Usually, herding groups of pastoralists claim traditional territories but seldom have scheduled grazing rights (Niamir, 1990). In the absence of legal protection in the past disputes were settled by force, and this prevails even today (Suttie *et al*., 2005).

In a system where animals are private property and land is not, there is always a tendency to keep excessive animal numbers and neglect pasture management, leading to over-stocking and over-grazing, causing serious damage to the vegetation that sometimes ends in desertification. Even if there are some grazing rules in existence they are not always respected. The lack of a feeling of ownership and responsibility in many African countries was a result of colonial regimes that tended to abolish all traditional rules without replacing them by adequate pasture management policies. Once traditional land-use rights were forgotten, their re-establishment proved difficult (Masri, 2001) if not impossible (Niamir, 1990). In an attempt to restore the productivity of mismanaged land, cattle ranches have been established with individual or group ownership of land, enabling herders to sustainably manage their pastures (Ng'ethe, 1992). However, this approach

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failed to understand that mobility is necessary to cope with the high variation in rainfall and fodder availability, and movement outside the delineated ranch area is necessary in excessively dry periods. Also, the ranch system proved a temptation for the stronger and successful herders to crowd out the less successful and to appropriate land at the expense of others (even to the benefit of non-agricultural profiteers).

Herds are often a mixture of cattle, sheep, goats, donkeys and camels. The preferred species is determined by the type of vegetation, water availability, topography, and distances to be travelled. Sheep, goats and camels are frequent where pasture resources are particularly scarce, bush dominates, and where long distances have to be covered daily to access drinking water. Cattle provide wealth and security but also meat and milk. They are often the dominant livestock species but during and after prolonged drought periods cattle numbers will be reduced as this species suffers most from the effects of insufficient rainfall.

Access to drinking water (together with feed) is one of the basic elements of pastoralism. Lifting of drinking water for livestock from wells is labour intensive. In fact, the labour available for this activity is often the main factor limiting herd size (Cossins & Upton, 1987). Because of migration of young family members to the cities, providing drinking water is increasingly a constraint. In northern Africa with less than 200 mm average annual rainfall, livestock was reduced after repeated prolonged droughts in the 1970s and 1980s, and the nomadic system was severely affected with losses of entire herds that never were replaced afterwards. Surviving sheep and goats replaced cattle and camels. When herders were forced to sell their remaining stock (to agriculturists, traders or government officers) and did not find job opportunities outside the system (Coppock, 1994), they were lucky to continue their profession as employed herdsmen (Fratkin & Roth, 2005). However, with diminishing grazing pressure the ecological threat seems to be reducing. Because of recent changes in the relationship with agriculturalists (see following paragraphs), many pastoralists can no longer sustain their herds because of increasing scarcity of dry season grazing.

Pastoralists practise subsistence systems of production, and livestock provides the basic livelihood of the families. Milk is the staple food of these people. Milk is obtained from cattle, sheep and goats but camel milk is particularly relished. Studies in eastern Ethiopia have shown average daily milk production from camels at 9.0 kg, from local cattle at 5.4 kg and from sheep and goats at 0.45 kg by nomadic pastoralists during the wet season (Degen, 2007). With non-Muslims in eastern Africa, animal blood has been a welcome addition mainly for the young warriors herding local cattle and goats, but this habit is disappearing. While milk supplies around 50% of energy to many pastoralist societies, some nomads live entirely on milk at least seasonally (Sadler *et al*., 2009). Energy is added to the protein-rich milk diet with the use of grains, either purchased or bartered. In addition, to a varying extent, pastoralists traditionally did grow some grain crops on attributed fields, for example wet pockets (depressions) in a desert surrounding where seasonally, during and after the rains, a crop could be raised. Some member of the family would stay at the fields during the cropping season. Alternatively, some of the extended families settle and completely engage in cropping (FAO, 2001).

If there is access to a market, pastoralists may even sell some excess milk and it is not uncommon for women to carry even small quantities over long distances to the market. If milk can be sold, depending on price relations, up to 16 times more energy can be obtained by purchasing grains with the proceeds. While the protein thus obtained may not have the same value as that in milk, undernourished children need energy primarily (Lynch, 1979). As individual milk yield is low (estimated at 252 kg per cow lactation) (Otte & Chilonda, 2002) and milk is rarely marketed, its importance for nutrition and household economy tends to be underestimated in nutrition statistics.

Pastoralists continue to dominate in the sub-Saharan zone experiencing 200–430 mm annual rainfall, where they keep about 24% of the total ruminant tropical livestock (Otte & Chilonda, 2002), but they are increasingly under pressure from agriculturists. Where grazing areas border cropping areas, rules are observed traditionally in order to mutually safeguard the individual interests of both pastoralists and agriculturalists. Grazing grounds are delineated where cropping is not allowed, corridors are established in cropping areas to ensure access of herds to water and pastures, and periods are fixed when stubble may be grazed after harvest. Procedures are established for compensation of crop damage caused by livestock. Conflicts between agriculturists and pastoralists may be caused by violation of these rules. Conflicts are fuelled by ethnic separation of agriculturists and pastoralists. Because of population growth, agriculturalists are encroaching on traditional grazing grounds, in particular those of higher precipitation, which served for dry-season grazing and retreat areas. However, grazing grounds are also lost by establishing erosion control belts, wildlife reserves and by afforestation. On the other hand, herders tend to invade cropping areas because of lack of rainfall.

Integration of animal husbandry (agro-pastoralism) with cropping is becoming more frequent in western Sahel countries, where traditionally both were strictly separated. This livestock keeping is more market oriented because for these people animals do not mainly provide security (Ndambi *et al*., 2007).

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1.3 FEED RESOURCES

Ruminants are equipped to mobilise energy and nutrients in grass and other cellulose-rich plant material to supply their needs for maintenance, including maintenance of body temperature and for movement, and reproduction together with nursing the young. Using ruminants for milk production is reasonable mainly if they feed on resources that cannot be used directly for human nutrition, such as grains and food produced on arable land. Use is made of their capacity to feed on grass and other roughages without competition for scarce human resources and convert it into milk (Preston & Leng, 1987). Ideally, these resource-conservative feeding systems are based on range and pasture, but in developing countries also on grazing waste land, stubble fields, roadsides, canal banks, fallow, tree plantations as well as feeding on straw and other agricultural by-products. This applies particularly to countries short on resources and which have difficulties supplying basic food to their population. Where resources are not in short supply and where there is a remunerative market for milk, feeding milking animals with grains (i.e. various kinds of animal feeds with higher concentrations of nutrients and energy than grass and other roughage, such as cereals, oil cakes and other by-products of the food processing industry), even in high amounts, may be economically justified (Speedy, 2001), whereas socioeconomically it still is criticised.

Nutrient requirements for milk production over and above pure maintenance are conveniently assessed by multiples of maintenance (Table 1.2). As can be seen in this table, even with a milk yield of only 2.7 kg daily, which is about the minimum to raise a calf, 1.2 times maintenance energy (i.e. 20% more) is required. Under tropical, scarce feed supply conditions, even cows of local breeds often have difficulties meeting their maintenance requirements and it can be deduced that there is little room for improved and more productive breeds. A production level of 13.5 kg milk per day requires a doubling of energy intake, which is hard to achieve under tropical conditions. It is possible only with more concentrated feeds such as improved pasture (grass and legumes, fertiliser, irrigation), cultivated fodder (e.g. alfalfa, maize) and grains in the diet.

Table 1.2. Daily energy requirements (MJ metabolisable energy) for maintenance and milk production.

Maintenance	Milk yield (kg)	Total requirement	Multiple of maintenance
60	2.7	72	1.2
60	6.7	90	1.5
60	13.5	120	2.0

Assumptions: Dairy cow, 359 kg liveweight, 0.75 MJ ME/kg body weight$^{0.75}$, 4.46 MJ ME/kg milk with 3.5% fat.

Source: based on data from National Research Council (2001).

1.4 ANIMAL SPECIES USED FOR MILK PRODUCTION

1.4.1 Cattle

Cattle are the predominant dairy species worldwide. They produce 83% of all milk (Table 1.3), comprising more than 90% in Europe and North America but only 75% and 60% in Africa and Asia, respectively. Milk from other species is statistically negligible in industrialised Western countries, although there are niche openings for mainly small but even big producers supplying specialised markets for gourmets and health-conscious consumers (e.g. from goats, sheep, camel, mares and donkeys). Unimproved cattle breeds of both *Bos indicus* and *Bos taurus* type, which are used mainly in multi-purpose systems for meat, milk, manure and draught purposes, are adapted to tropical conditions by virtue of heat tolerance, disease resistance, better feed intake and digestion of low-quality feeds. They produce quantities of milk sufficient to raise their young, possibly even twins, but some additional milk in excess of the calf's need may be extracted from the cows mainly for improving the family diet while small quantities may also be marketed.

In order to qualify as a dairy breed, cattle and other species must be able to produce milk well in excess of the neonate's requirements and in addition must yield their milk to humans uninhibitedly at milking rather than by simultaneous presence or suckling by the young calf, kid, lamb, etc. Among the many hundreds of breeds listed globally, there are only a few of worldwide dairy importance (Mason, 1996).

All milk-producing animals, with exceptions where religious taboos exist, are eventually used for meat. Male calves may be a valuable asset, adding income to the dairy enterprise, but not in all production systems (see section 1.2.2). In tropical countries, almost all traditional production systems are dual (multiple) purpose, where meat and draught power are other benefits along with milk. However, the concept of dual-purpose breed would require special attention to be given to meat characteristics in selective breeding.

With few exceptions, dairy breeds comprise *Bos taurus*. There is a tendency towards a few highly selected and productive breeds for worldwide distribution, such as the Holstein-Friesian. The concentration on a few breeds and often on few pedigree lines within breeds is raising concern of possible inbreeding depression and loss of genetic diversity.

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Table 1.3. World milk production (tonnes) 2009.

	World	North America	South America	Europe	Africa	Asia	Oceania
Cow	580 481 508	94 074 260	59 179 319	208 947 600	27 646 809	150 187 182	24 671 234
Buffalo	90 333 830			217 192	2 640 638	87 476 000	
Goat	15 128 186	160 000*	182 440	2 468 861	3 206 195	8 909 416	40
Sheep	8 974 689		35 670	3 053 751	1 790 384	4 094 883	
Camel	1 636 132			80	1 456 107	179 945	
Total	696 554 346	94 074 260	59 397 429	214 687 484	36 740 134	250 847 426	24 671 274

*There are no statistics available on US goat milk production. National Agricultural Statistics Service (2011) estimates total number of milking dairy goats at 232 000 in 2011; if they produce 600 kg saleable milk yearly (Milani & Wendorff, 2011), then 139 000 t may be the annual production. Goat milk production in Canada was estimated at over 21 000 t in 2004 (Agriculture and Agri-Food Canada, 2006).

Source: based on data from FAO (2011b).

Table 1.4. Lactation* milk yield of dairy cattle breeds (based on data from herd-book averages, 2009).

Breed	Yield (kg)	Fat (%)	FCM†
Black and White, USA‡	10 510	3.64	9 942
Black and White, Israel§	11 903	3.52	11 046
Black and White, Germany¶	8 887	4.07	8 980
Brown Swiss, USA‖	8 673	4.06	8 751
Jersey, UK**	5 673	5.39	6 856

*As length of lactation varies, a standard lactation of 305 days' duration is generally recorded.

†FCM, fat-corrected milk allows comparison of milk yield with different fat content. FCM = 0.4 M + 15 F (M, milk yield; F, butterfat yield; all in same units, e.g. all as kg).

‡http://holsteinusa.com/holstein_breed/breedhistory.html

§http://www.israeldairy.com/info/dairy-farming/annrep2008.pdf

¶http://www.holstein-dhv.de/leistung.html

‖http://www.brownswissusa.com/documents/annual_report/09annualreport.pdf

**http://www.ukjerseys.com/breed/cdi/cdi_2008.pdf

1.4.1.1 Milk yield

The economically most important trait is the per-cow annual or lactation milk yield. It depends on daily amounts of milk and length of lactation. Length of lactation is quite variable. Milk secretion ceases when the cow is pregnant again, which may happen after 3 months, but under less favourable conditions much later. In modern dairy breeds, the cow is expected to be pregnant again 6 weeks after birth. Under normal conditions, the milk secretion of pregnant cows gradually decreases and milking is discontinued about 6 weeks before the next parturition (Svennersten-Sjaunja & Olsson, 2005). Udder secretion after parturition and following a dry period is called colostrum. Its composition differs from later milk and it is indispensable for the calf, as it conveys antibodies and essential nutrients to the calf. It is not considered milk for human nutrition according to food legislation in most countries. However, it is relished in some cultures and for special products and health formulae. Some cows, the high-producing ones in particular, may voluntarily continue lactating even until the following parturition. In order to allow a sufficient rest period and for formation of the colostrum, cows are intentionally dried off after 10 months of lactation. Consequently, lactation records are usually standardised at 305 days in order to exclude effects of different lactation length. Average lactation milk yield per cow is between 4000 and 7000 kg in most developed countries but has attained remarkable levels with highly selected breeds and in high-input systems of production. The yield of major breeds is shown in Table 1.4. The average lactation yield of all dairy cows in the USA

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was 9601 kg in 2010 (USDA, 2011). The top-producing 133 Holstein herds had an average annual yield of 13 368 kg (Kellog *et al*., 2001). With her very special economic situation, Israel excels over all other countries. Growth hormone (bovine somatotropin, BST or BGH) stimulates milk secretion, and can be synthesised and administered to cows for increasing milk yield. However, because of health concerns, both in cows and milk consumers, this practice is banned in most milk-producing countries except the USA (European Commission, 1999a, b).

1.4.1.2 Milk composition

Milk composition varies greatly between livestock species, and between breeds to a lesser extent (Table 1.4). It is often observed that milk from *Bos indicus* or other species is superior in composition, and fat content in particular. However, there is a general tendency for milk to be more concentrated when low in quantity and during the course of the lactation: as quantity declines, component concentrations increase (Pirchner & Nibler, 2000), so that differences in fat content tend to disappear when milk production is compared at an equal milk yield basis and only full lactation data are useful.

In addition, some components like butterfat are modified by feeding. Therefore, values of the main components serve only as an indication of approximate differences between species. The data in Table 1.5 are extracted from several sources in the literature and based on personal observations. Over time, specific milk constituents have received different consideration. With cattle in particular, breeders have tried to elevate fat and protein content, especially where cheese production from milk is of major interest. However, high fat and protein percentage is not economically important under all conditions. Milk plants may pay little or no attention to differences in fat content if they market mainly fluid milk. However, if milk is mainly processed into products, the price paid to the producer is generally based on the fat and often protein content. Therefore, breeders aimed for high fat and protein content, although this was a misconception as it was the quantity of fat that producers were paid for, which is determined by both fat content and milk quantity. In Europe in particular, for a long time the goal was a minimum of 4% fat. This changed only when creameries included volume as a negative factor in their price formula. Surprisingly, in North American Holstein-Friesians where less attention was paid to fat, average fat content was not much lower (Table 1.5). Also, the negative relationship between milk quantity and fat content (Pirchner & Niblet, 2000) has to be taken into consideration when trying to increase fat percentage by selective breeding. In areas where milk is processed to butter or ghee, Jerseys or water buffaloes (breeds with high milk fat) are advantageous, whereas city fluid milk dairies prefer a high proportion of high-yielding dairy breeds even if their milk has low fat content.

1.4.1.3 Milk production in the tropics

Milk yield is generally low in the tropics because of insufficient nutrient supply, husbandry conditions (milking technique, suckling of calves) and genetic disposition. The main cause of reduced milk yield under heat stress is reduced feed intake, but direct metabolic effects of ambient temperature may also be involved (Rhoads *et al*., 2009).

In traditional systems where local cattle are milked while the calf is sucking, the annual saleable milk production per cow may be only about 1000 kg or less (Preston, 1991), but with improved local breeds it may be as high as 3000 kg, and even more with intensive production conditions. Milk production per hectare and year on natural pastures is about 1000–1600 kg. On grass and legume pastures it may reach 5000–9000 kg. On intensively fertilised and irrigated pastures it might be even higher (Trujillo, 1991). When comparing milk yield between animals in the tropics the calving season has to be taken into account because of seasonality of feed supply and its influence on milk yield.

Understandably, breeders in the tropics have tried to increase milk production with dairy breeds of European origin. In general, the performance of specialised dairy breeds of *Bos taurus* in the tropics lags behind that in temperate environments, mainly because the feed requirements of large cattle can hardly be met by smallholders (Preston, 1991).

Table 1.5. Milk composition (%, averages and ranges) of cattle, buffalo, camel, goat and sheep.

	Cattle	Buffalo	Camel	Sheep	Goat
Dry matter	13	17–19	7.0–10.7	16–20	11.5–13.5
Fat	3.4–5.4	7.0–8.5	2.9–5.4	5.0–8.0	3.5–8.0
Protein	3.5–4.0	3.6–4.6	3.0–3.9	5.0–6.5	2.8–3.0
Lactose	4.6	4.6–5.0	3.3–5.8	4.4	3.9–4.4

Source: based on data from Park & Haenlein (2006, 2010).

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As a consequence, growth rate, mature weight and fertility are generally low, and calf losses and mortality rate are high. This is accentuated by low feed quality and poor animal management. However, with adequate feed and management, milk production can be increased by crossbreeding local cattle with European dairy breeds (Cunningham & Syrstad, 1987). However, the proportion of dairy breed genes in crossbreeding must not exceed 50%, otherwise adaptation to tropical conditions will be compromised.

Under favourable tropical environmental and husbandry conditions, in tropical highlands in particular, dairy breeds can be kept successfully. More intensive forms of dairy production with adequate nutrition and management, including sometimes cooling with fans or water and low disease challenge, frequently achieve high milk yields, for example Holstein-Friesians in California, Arizona, Israel, Italy and Mexico (Table 1.4) and in peri-urban herds around many tropical capitals (de Leeuw *et al*., 1999). However, the economics of such operations depend largely on input–output cost relationships.

While most of the milk worldwide is produced by *Bos taurus* breeds, some is still produced with local, mainly *Bos indicus* and crossbred cattle. In tropical and subtropical Asia the majority of cattle comprise *Bos indicus* (Zebu, Brahman). They are kept mainly for draught, meat and manure. Characteristic traits include a hump, large dewlap and sheath fold, large hanging ears, sloping pelvis, fine legs, fine and smooth hair but, most importantly, heat tolerance and tick resistance (Berman, 2011). Examples of *Bos indicus* dairy breeds include the Red Sindhi, Sahiwal, Gir (Stonaker *et al*., 1953), Kankrej, Rath and Tharpharkar in India and Pakistan, Guzerat and Gir in Brazil, and Fulani in western Africa (Madalena, 2002).

Mainly because of their adaptation to the tropical environment they are also used outside their Asian area of origin. Milk yield can be low in most Zebus and milkability is not efficient. Sucking by the calf prior to milking or at least presence of the calf at milking, is necessary with most Zebus in order to achieve sufficient milk let-down and milk flow. Milkability is related to persistence of daily milk yield, and cows lacking good genetic dairy characteristics may cease lactating as early as 100 days after parturition. However, some Zebu cows handled very carefully may be milked even without the calf being present, at least after the first weeks of lactation. Also, Zebus respond to genetic selection for milk yield, milk composition, udder conformation and milkability (Hayman, 1977). There are important populations of Guzerat (Peixoto *et al*., 2006) and Gir (Gaur *et al*., 2003) in Brazil that have been developed to be productive dairy animals by pure breeding, and of Sahiwal in Kenya, Africa (Trail & Gregory, 1981). Average 10-month records in Jamaica for Sahiwal Zebus have been reported at 2185 L over 260 lactations, for Fulani Zebus at 756 L over 1030 lactations (McLaren, 1972). Average lactation yields of six Zebu populations in India ranged from 1403 to 1931 kg for a lactation length of 257–351 days; for 27 431 Gir and 2298 Guzerat Zebus in Brazil, 2278 kg and 2400 kg for a lactation length of 291 and 285 days, respectively; and for 17 292 Sahiwal Zebus in Pakistan, 1522 kg for a lactation length of 256 days (Madalena, 2002).

1.4.2 Sheep and goats

Worldwide, about 3.5% of all milk is produced by goats and sheep, both referred to as small stock. This term relates not only to the size of these animals, but also to a notion of their value. Worldwide there is a tendency to value cattle higher than small stock and this reflects on the social status of the owners. However, in some areas small ruminants are valued for their specific products, and are kept even when cattle husbandry is possible or practised. Examples of this kind of small ruminant husbandry can be found in:

- France, Italy, Spain, Portugal, Greece, Norway and some other European and Mediterranean countries where milk for cheese processing is produced by sheep and goats, sometimes in intensive systems;
- Near and Far East, where milk and dairy products from sheep and goats are preferred over those from other animals;
- Islamic countries, where lambs and kids play an additional important role in religious feasts and holidays. Jewish and Islamic populations do not eat pork, while Buddhist populations do not eat beef from European (*Bos taurus*) breeds.

Goat and sheep milk is mainly processed into cheese and fermented products (yoghurt). Sheep breeders were the first in France to obtain the label 'AOC' (Appellation d'Origine Contrôlée) for Roquefort cheese (Ministère de l'Agriculture et de la Pêche, 2001). The Confédération Générale des Producteurs de Lait de Brebis et des Industriels de Roquefort successfully defends the label with a strong legal department (Roquefort 2011). Another 10 sheep and about 20 goat cheeses have obtained the EU label 'Protected Designation of Origin' (Designation of Origin, 1999) and thereby secured market advantage. Also in Europe, goat breeders benefit from the fact that goat milk production is not controlled by the quota system (see section 1.13).

Small ruminants are very adaptive and can stand both cold and hot climates. Small ruminants require only limited resources. In developing countries, in small herds they contribute to the sustenance of poor families and the supply of local markets, and are a way of investing surplus cash

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Table 1.6. Milk yield of goats and sheep.

	Milk yield (kg)	Fat content (%)	Days of lactation
Goat[1]	160–1900	3.5–8.0	305
Sheep[2]	160–600 (900)	5.3–9.3	270
Sheep, Improved Awassi[3]	506		214

Sources: based on data from [1] Park & Haenlein (2010); [2] Haenlein & Wendorff (2006) (900 kg were recorded in a 365-day lactation period); [3] Gootwine & Pollott (2000).

from cropping. Because the animals can be readily sold, the capital is available at any time. Interest accrues through growth and reproduction, although the risk (loss due to disease, death, predators or theft) may be high.

Small ruminants can be kept on extensive range with scarce feed supplies as well as intensively with high feed input. They utilise pasture, fodder and shrubs that are not suitable for cattle, such as on mountain ranges, steeply sloping land, dry steppe and desert, and marginal, residual and fallow land, and can utilise agricultural by-products. Small ruminants are not usually kept as the sole livestock in many countries, except for desert areas; more often they are kept with cattle and other species by the same owner.

Some goat breeds are true single-purpose dairy animals, especially the so-called Swiss goats, the Saanen, Alpine, Toggenburg, and Oberhasli, besides the La Mancha, Manchego, Nubian, etc. In relation to their body size and feed intake, goats equal dairy cattle even when compared at high production levels (Table 1.6). The milk yield of a 65-kg goat (about 1000 kg lactation total) equals that of a 680-kg cow (6100 kg lactation total) when compared on the basis of metabolic body weight, because metabolism is not related to body mass linearly but is proportional to the ¾ power of body mass. Also, the energy requirements to produce milk are about the same. In addition, the milkability and lactation persistence of goats can be excellent and mechanical milking is practised in many countries. The best dairy goat breeds are of Swiss origin. Some of them have been used worldwide to improve local breeds. Best known is probably the Saanen, which attains herd yield averages of 1000 kg per lactation. Using genetic selection for milk yield, individual dairy goats in the USA have achieved daily production levels of 6–12 kg with twice daily milking, producing up to 3620 kg over a 305-day lactation (Haenlein, 2007). On a 4% FCM (fat-corrected milk) basis, the records were 2380 kg for a La Mancha, 2438 kg for an Oberhasli, 2506 kg for a Saanen, 3150 kg for a Nubian, 3266 kg for an Alpine, and 3578 kg for a Toggenburg, approaching or equalling the highest producing Holstein dairy cows.

Similarly, some sheep breeds have achieved fairly high milk yields (Table 1.6). The normal lactation period is however only around 200 days. Although their milkability, especially their udder conformation, does not yet equal that of cattle or goats, technology exists for efficient mechanical milking. There are two excelling breeds, the East Friesian milk sheep and the improved Israeli Awassi, which produce on average 500–600 kg per lactation, and some dairy sheep have produced more than 1000 kg per lactation, which on a total solids basis equals that of high-producing dairy goats (Haenlein, 2007).

1.4.3 Buffalo

Buffalo (water buffalo, *Bubalus bubalis*, not to be confused with the American bison, *Bison bison*, which is commonly referred to as buffalo) are widely used as dairy animals in Asia, India and Pakistan in particular, where their milk is highly valued. Probably more people depend for milk on water buffalo than on any other livestock species in the world (Kumar *et al*., 2006), although the same can be claimed for goats but reliable statistics do not exist. Buffalo milk is preferred over cow milk because of its taste and high fat content. Buffaloes are also kept in some Latin American countries (notably Brazil, Argentina and Venezuela), and there are buffalo populations in Egypt, eastern Europe (Bulgaria, Romania and the former Yugoslavia), Iran, Iraq and Turkey. In Campania, Italy, buffaloes are kept in a shed under intensive management conditions for the production of Mozzarella di buffalo cheese, which is protected by the Denomination of Controlled Origin (DOP) (European Union, 2008). It is defended by the Consorzio di Tutela della Mozzarella di Bufala Campana (Repubblica Italiana, 2011). Buffalo milk does not fall under the EU quota system.

The buffalo is well adapted to the hot tropical environment but needs shade or wallow during the heat at noon. It consumes and converts fibre-rich roughage, such as straw. Milkability is limited and the presence of the calf is necessary in order to stimulate milk let-down, at least during the early part of the lactation, but breeds and technological

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Table 1.7. Milk production of water buffalo.

Country/breed	Body weight (kg)	Milk (kg)	Fat (%)	Lactation length (days)	Calving interval (days)
India, Murrah	495	1800	7.5	305	479
India, Nili-Ravi	546	2000	6.5	305	443
India, Pandharpuri		1142	7.0	305	
India, Surti	550–650	2090	6.6–8.1	350	510
Pakistan, Nili-Ravi	625	2070		312	
China*		2262		316	
Egypt†	300	1850		180	
Italy	650	2221	8.1	270	

*Mainly Murrah and Nili-Ravi.

†Borghese (2010).

Source: based on data from Borghese (2005).

systems exist where milking machines are applied. There are many breeds in Asian countries, with nine well-described breeds in India alone: Bhadawari, Jaffarabadi, Mehsana, Murrah, Nagpuri, Nili-Ravi, Pandharpuri, Surti and Toda. Liveweight is between 500 and 600 kg (Table 1.7). Milk yield during a 270–350 day lactation, followed by a 140–300 day dry period, is between 500 and 2100 kg per lactation (often exceeding that of local cattle in tropical countries) with 4.5–8.6% fat content. Under intensive management in Italy, milk yield achieved up to 5061 kg with 8.6–10.3% fat in a 270-day lactation (Rosati & vanVleck, 2002).

1.4.4 Camel

The Arabian or one-humped camel (*Camelus dromedarius*) is an important milk animal in pastoral systems in semi-arid northern and north-eastern Africa (Sahel), on the Arabian Peninsula and the Indo-Pakistan subcontinent (Rajasthan in particular). Many people in these areas seasonally depend on camel milk and take a specific liking to the camel, which plays an almost mythical role in their lives (Lhoste, 2004). Camels are kept mainly where environmental conditions (temperature, water availability, feed quality) do not favour cattle-keeping. Here, they may produce more milk than cattle. Camels are milked in the presence of the calf. As the cistern volume is very small, frequent milking is necessary (three to four times daily). Milking begins typically 3 months after parturition and may continue for 12–18 months. Camels continue lactating even when water availability is restricted (Bekele *et al*., 2011). Milk yield varies depending on environmental conditions (Faye, 2004). Data in the literature vary greatly and are difficult to compare as conditions influencing records are not always stated (frequency of milking, milk suckled by the young included or not, length of lactation, calving interval, feeding, field or experimental data). Average daily yield of 1–2 kg and 1000–1500 kg per lactation (Kaufmann, 1998) can be expected but with feed supplementation 6 and even 12 kg per day have been reported.

There is a luxury market on the Arabian Peninsula and in North African countries. Some intensive camel dairies benefit and produce close to urban centres. Although the main herd continues under pastoral management, some lactating camels are fed intensively for about 12 months and milked with machines, with milk let-down sometimes stimulated with injections of oxytocin, the hormone that causes milk to pass from the secretory tissue into the holding cistern of the udder (Balasse, 2003).

The double-humped camel (*Camelus bactrianus*) is adapted to both the extreme hot and cold climates of northern deserts and is kept in transhumance systems mainly in central Asia's steppe regions. Although kept mainly as a pack animal and producer of fine wool, it is also milked. The milk is a traditional staple food, especially in Mongolia (Gobi desert). Average milk yield during an 18-month lactation is reported to be 174–576 L (Saipolda, 2004) but can reach 15–20 kg daily during peak lactation and 1000–1500 kg in a 305-day lactation (Baimukanov, 1989). The milk is used to make butter, cheese, curd, yoghurt and other fermented products.

1.4.5 Mare

Traditionally, mares are milked in some central Asian countries: Mongolia, Kirgisia, Kazakhstan, Kyrgyzstan and Byelorussia. Mares' milk is an important asset for pastoral people, accounting for about 8% of all milk produced. Along with other exotic livestock, mares are kept for milk in many industrialised countries, producing mainly for the

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fad or health food market. Many breeds are milked but heavy horses are preferred in specialised dairies, where in Europe the Haflinger breed with its excellent milkability characteristics is more frequently used (Zollmann, 1985; Doreau & Boulot, 1989; Park *et al*., 2006).

Mares are usually hand milked in the presence of the foal to stimulate let-down, at least at the beginning of lactation. In specialised operations machine milking is practised. Frequent milking (more than twice daily) is necessary because the cistern volume is small. Under natural conditions mares nurse their foals for up to 12 months, while milking lactations last for about 6 months, sometimes even 9 months. The mare's average daily milk yield is 10–15 L (Doreau & Boulot, 1989). Annual milk production (generally in a 6-month lactation) can be 1500–2560 L of marketable milk (Kosharov *et al*., 1989). Milk is mainly processed into fermented milk products; Koumiss, popular in Russia and Asia; Airag in Mongolia, results from some alcoholic fermentation.

1.4.6 Yak

The discussion in this section relies heavily on Wiener *et al*. (2003/2006). The taxonomy of the yak is not quite clear, but there is a tendency to classify it as a species (*Poephagus grunniens* or *Bos grunniens*) of the genus *Poephagus* (belonging together with *Bos* and *Bison* to the Bovinae; Olsen, 1991). With 60 chromosomes, the same as *Bos taurus* and *Bos indicus* and *Bison*, the yak interbreeds with both; the female offspring are fertile but the males are not (Deakin *et al*., 1935).

Yaks are adapted to low temperatures, high altitude (low oxygen pressure), high solar radiation and scarce vegetation. On their own, they prefer grazing at altitudes between 4000 and 6000 m above sea level. Yaks are typically husbanded between 2500 and 5500 m, mainly above the treeline, with cool moist summers and severely cold winters. There is frost all year round with a very short growing season. Yak husbandry is part of the social and cultural life of the people living at these inhospitable altitudes.

Yaks are kept in the Himalayan mountain range, predominantly on the Qinghai–Tibetan Plateau and other regions around the Himalayas (Wu, 2003/2006), where many prosperous pastoral groups still exist (Sarbagishev *et al*., 1989) Yaks are also kept in the high-altitude areas of the republics of central Asia, mainly in Kirgisia, Tajikistan, northwest China, Mongolian People's Republic, Nepal and Tibet. Small numbers are kept in India, Bhutan, Afghanistan, in the north Caucasus and in southern Siberia and Yakutia.

Yaks in Mongolia are kept in a pastoral transhumance system, herds alternating between low (cold season pasture) and high mountains (warm season pasture), but recently more herders have settled. Although this has merit in providing an infrastructure for the community and raising the standards of social services for yak herders, it entails the problem of land degradation (Wu, 2003/2006). The yak is not a dairy animal but traditionally herders take milk for domestic consumption and milk is the most important of the yak products. The yak is milked in the presence of the calf. Yield is estimated between 1 and 3 kg daily during the five summer months. Fat content is 6–7% (Dong *et al*., 2007). Milk is consumed fresh or processed into butter, fermented products and cheese.

1.4.7 Reindeer

This section is based on information from Holand *et al*. (2006) and Vistnes *et al*. (2009). The reindeer (*Rangifer tarandus*), or caribou in North America, is an arctic and sub-arctic deer. Reindeer herding can be dated at least as far back as the late Iron Age. Reindeer are herded by Eurasian arctic and sub-arctic people including the Sami, also known as Laps (in Norway, northern Sweden, and neighbouring Russia), the Nenets (in the polar regions of north-east Europe and north-west Siberia) and the Inuit (in Canada and Siberia). Traditionally, reindeer herders migrate with their herds between coast and inland areas following annual routes. Reindeer are raised in the taiga and tundra for their meat, hides, antlers, transportation and, to a lesser extent mainly in the taiga, for milk. They are the only source of milk because no other milk animal can live in these zones. Milk is consumed fresh or processed. There is evidence that milking reindeer was important for the northern nomads but it was abandoned early in the twentieth century except in south-eastern Siberia and Lapland (Holand *et al*., 2006). Reindeer are not fully domesticated and do not breed in captivity, but they were tamed for milking in northern Norway/Lapland. Average milk yield is between 100 and 500 g daily, with about 100 kg per lactation.

1.5 BREED IMPROVEMENT

Animal husbandry is inextricably related to selective breeding. All those characteristics necessary for environmental adaptation, survival, reproduction and population growth have developed with evolution, whereas the traits necessary for purposeful production had to be increased with domestication. In the case of milk production these were milk yield and milkability, but also all predisposing traits like docility, precocity, reproductive rate and feed intake. The quantity of feed needed for high levels of milk production requires an animal that is eager to feed plentifully. In selecting for all these productive traits the original adaptive traits must be conserved (Menjo *et al*., 2009).

This may seem self-evident, but with increasing protection of livestock (housing and healthcare), improved nutrition,

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and controlled reproduction and rearing, adaptation to adverse environmental conditions (as in the original local breeds) is not always conserved. As can be seen from Table 1.4, the better dairy cattle populations are presently producing around 10 000 kg per lactation. With advanced breeding methods it is not difficult to provide the breeder with the appropriate genetics. However, as these very high levels of milk production have been attained, renewed attention is being given to adaptation and traits which will ensure that healthy animals stay in the herd for many lactations (Groen *et al*., 1997; Gay *et al*., 2011; see also section 1.13).

Livestock breeding is beset with the problem that not all desirable traits can be combined. Some traits may even be linked with undesirable characteristics (Clark, 1998). Examples of antagonistic traits in dairy cattle include early maturation and longevity (Essl, 1998), milk yield and fat content, milk yield and reproductive efficiency, and milk yield and meat production in dual-purpose cattle. Careful economic evaluation and giving appropriate weight in selection indices can tackle the problem (Pearson & Miller, 1981).

1.5.1 Pure breeding

With the inherent low reproductive rate of cattle, the potential to select among cows is limited and breeding efforts concentrate on sires. Traditionally in the past, sires were selected on the basis of their dam's quality and on physical appearance. Although all the physical traits of an animal (phenotype) result from gene action (genotype), the progeny of superior individuals does not necessarily exceed the average population. Thus, selection on the basis of individual merit understandably yields only slow progress. Attempts to assess a sire's breeding value on the basis of daughter performance can already be seen in the eighteenth century but systematic science-based methods to estimate breeding value began only in the twentieth century. As genes are transmitted to the progeny randomly, large numbers of progeny are necessary for reliable estimates (Lush, 1937). Pure breeding has dominated the development of the superior European dairy cattle breeds and crossbreeding has not been practised widely, except in the tropics.

1.5.2 Artificial insemination

Substantial progress in breed improvement came with the advent of artificial insemination (AI) (van Vleck, 1981). Because with AI one sire could produce thousands of progeny, the number of sires needed was much less and they could be selected much more rigorously. In addition, expensive shipping of live breeding stock was obviated and worldwide gene transfer was facilitated. Today, European dairy cattle are almost exclusively bred through AI and the breeding value of all sires is estimated with elaborate methods and a high degree of accuracy, which allows their ranking for expected progeny performance (Pearson & Miller, 1981).

AI is much less common in other dairy species, although practised to a limited extent in goats, sheep, buffalo and even the mare. Even in tropical developing countries AI is regularly applied in dairy cattle. However, the elaborate system required is not always available and deficiencies in the system may cause low conception and calving rate.

1.5.3 Embryo transfer

Transfer of embryos (following stimulation of multiple ovulation) into foster mothers (multiple ovulation and embryo transfer or MOET) makes it possible to obtain from outstanding dams more progeny than they could raise naturally. It also means much lower transportation costs in the export business. This led to further improvements in genetic progress by more accurate and intense selection and shorter generation intervals (Teepker & Smith, 1990).

1.5.4 Genomic selection

Recently, additional selection response is being achieved by genomic section (Hayes *et al*., 2009; Goddard *et al*., 2010). Following sequencing of the bovine genome, many DNA markers in the form of single-nucleotide polymorphisms (SNP) have been discovered. Genomic breeding value (GEBV) is computed using a reference population of animals that have high-density genotype as well as phenotypic information (de Roos *et al*., 2011; Weller & Ron, 2011). The genomic breeding value can be predicted at birth, thus largely enhancing genetic gain by reducing the generation interval.

1.5.5 Crossbreeding

Genetic improvement through selection for milk yield within breeds is a tedious long-term process. Therefore, breeders often take to crossbreeding (Taneja, 1999). Crossbreeding was frequent in the early stages of developing a breed. This can be a single introduction of a certain trait not present in the original breed but available in another breed by using one or a few sires for one or a few generations followed by selection for the new trait in subsequent generations. Alternatively, two breeds can be combined to form a new breed which carries desirable traits of the two. Though often claimed, the superiority of individual dairy breeds for crossbreeding is insufficiently proven (choice of breed very much depends on experts' personal experience with a particular breed). However, the use of well-established breeds with large populations in their homeland is to be preferred for organisational reasons. Thus, Black and White cattle (Holstein-Friesian), as

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also Saanen goats and East Friesian dairy sheep, have been used worldwide for crossbreeding.

In tropical countries where local cattle are poor milk producers, it was often tried to increase their milk yield by using European dairy breeds. However, because these European breeds are insufficiently adapted to the environment, they perform well only with high inputs. Therefore, European breeds are crossed with local breeds so as to produce mainly half or three-quarter crossbreeds (i.e. containing half to three-quarters of the genes of dairy breeds) (Mason & Buvanendran, 1982). Provided feeding and management are adequate, the milk yield of these crosses can be satisfactory. This process is difficult for smallholders, and there is the problem of maintaining the appropriate gene proportion. In addition, there is the danger of valuable genetic resources being lost. Therefore projects for increasing milk production by genetic improvement often failed.

Several synthetic breeds were formed by crossing *bos taurus* with *bos indicus* cattle in order to sustainably combine the dairy characteristics of temperate breeds with the adaptation (heat tolerance, disease and tick resistance) of tropical breeds (Wellington & Mahadevan, 1977; Taneja, 1999; Madalena, 2002). However, among many attempts only a few were sustainable (Mason, 1996). Examples of successful programmes are crosses between Zebus and dairy breeds in Jamaica and Australia. The Jamaica Hope was developed with Jerseys and Fulani Zebus. Howe (1949) had shown that crossing with Zebus could improve growth rate, milk yield, milk fat content and reproductive efficiency of *bos taurus* cattle. Milk yield of 269 Zebu-Jersey crossbreed lactations was 1489 L, and of Zebu-Friesian crossbreeds 2143 L over 77 lactations (McLaren, 1972). The breed was officially recognised emphasising their heat tolerance, and fertility combined with high milk yield (Lecky, 1951). A breed society was formed with about 50 members. From 1950 to 1964, milk yields of 2153 305-day lactations averaged 2676 kg (McLaren, 1972). While some Jamaica Hope cattle were exported to Caribbean and Latin American countries, the breed has not experienced widespread use. Dairy producers apparently prefer the more productive Holstein-Friesian benefiting from support by a strong breeders association. The extra cost for management and health care is offset by additional income. The Australian Friesian Sahiwal (AFS) contains 50% each of *b.taurus* and *b. indicus*. Under good Queensland conditions it produced 2749 kg milk and 115 kg fat against 3670 kg and 141 kg by Holstein-Friesians. But under wet tropical conditions AFS milk and fat yield excelled over the Holstein-Friesian by 124 and 141%, respectively (Taneja, 1999). The Australian Milking Zebu (AMZ) was developed with 20 to 40% *Bos indicus* (Sahiwal, Red Sindhi) and 60 to 80% Jersey. Milk and fat yield of AMZ (3304, 146 kg), Guernsey (2913, 124 kg) and Friesian (4165, 138 kg) were comparable under favourable environmental conditions. But AMZ excelled in heat tolerance: exposure to 36–40.5°C reduced milk yield by 30% in Friesians but less than 5% in AMZ (Hayman, 1977). Similar crossbreeding efforts have been made in Venezuela and Cuba (Madalena, 2002).

1.6 NUTRITION

Herbivores, as the term implies, live on plant material. The preference of livestock species for plant families differs. Some select nutrient-rich, easily digestible matter (e.g. sheep and goats) while others content themselves with fibre-rich roughage of lesser digestibility and nutrient density (e.g. buffalo). However, all are able to metabolise plant nutrients into milk. Efficiency of feed net energy conversion into milk is very similar in all species but depends largely on the energy density of the feed (Van Soest, 1994). Traditionally, all herbivore livestock were allowed to graze. Where environmental conditions did not allow year-round grazing, hay or silage was prepared for winter feeding. With good feeding management a cow can produce about 4000 kg milk per lactation on the basis of quality roughage alone (grass, hay, silage). With increasing production, feeding of concentrates becomes necessary. These will add protein and energy. As a rule of thumb, 1 kg concentrate will provide nutrients for about 2 kg milk. The feed requirements of high-producing cows are remarkable and it needs a cow willing to eat great quantities and a herdsman able to handle the art of prudent feeding. A cow of 454 kg liveweight producing 30 kg milk will have to eat as much as 13 kg dry matter daily (National Research Council, 2001). In order to produce 8000 kg per lactation, a cow will need in addition to roughage about 2000 kg concentrates. As long as the price relation between milk and feed are favourable, even feeding such high quantities of concentrates can be economical (Williams *et al*., 1987).

For proper functioning, the rumen needs a large microbiota population to ferment the dietary fibre, so feeding such quantities of concentrates (containing little fibre) can easily disturb the fragile equilibrium of the microbiota in this dynamic organ. Depending on the mineral (and micronutrient) content of feeds, it may be necessary to supplement the rations with these substances. Supplying a balanced diet with all the nutrients (protein and energy but also minerals and micronutrients) to meet the demand for production without provoking metabolic disorders is a challenge for the dairyman.

In the twentieth century, keeping cows in confinement year round and feeding maize silage (supplemented with protein) became the predominant system, mainly because higher yields per hectare were achieved and herd management

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was simplified. Alternatively, alfalfa (or grass) may be the main roughage; in this case, energy must be supplemented. However, recent research has indicated that the healthier pasture grazing system, even though producing less milk yield, may fare better economically than production in confinement (White *et al*., 2002).

What has been said about cattle applies *mutatis mutandis* to other species as well but high feed input may not be appropriate with all species and in all production systems, unless genetic ability has been provided by selection.

1.7 ANIMAL HEALTH

Many livestock diseases can impair animal well-being, reproduction and production. Physical injuries and fractures are not specific to dairy animals but joint injuries and distortions may be caused by inadequate housing conditions, for example a slippery loafing area in free-housing (Webb & Nilsson, 1983). Infectious and transmissible (bacteria, virus) diseases as well as endo- and ecto-parasites are a menace wherever animals are kept in great numbers. Crowding animals in houses and on pasture increases the risk of disease transmission (Lean *et al*., 2008). Youngstock prior to weaning are particularly susceptible. Blood parasites transmitted by insects and ticks are a great problem in the tropics to the extent that some areas where these are endemic may not be usable by livestock except for resistant breeds and species (e.g. trypanosomiasis in Africa) (FAO, 1992), or continuous expensive preventive measures may be necessary.

Considerable effort is necessary to prevent negative effects and keep livestock healthy. The importance of disease varies with animal management (nutrition in particular) and environmental conditions. A hot and humid tropical climate favours diseases but in the tropical highlands disease pressure is much less and even high-producing dairy animals may be kept.

High-yielding dairy animals are particularly prone to diseases. Metabolism, reproductive functions and the mammary gland are under heavy stress. Special care is therefore required to protect these organs and systems from negative effects or even from collapse. Although disease incidence in herds with fewer than 100 cows has been found to be less than in larger herds, the risk of mortal disease does not seem to be increasing with herd size (USDA, 2007). Among 30 endemic livestock diseases, mastitis causes the highest economic cost followed by lameness (Bennett, 2003). In a selection experiment where two lines were established, with average and high milk yield (5753 vs. 6693 kg per lactation, respectively), it was shown that the high yielders incurred higher cost for mammary problems but less cost for reproductive problems (Dunklee *et al*., 1994a, b). Thus, careful herd management and close surveillance can check the impact of disease even in large herds with high levels of production. However, the total cost of diseases (over and above direct veterinary expenses) is difficult to assess, but is reflected largely in the cost of reduced lifetime production (see section 1.13).

Immunisation (vaccination) is possible against various infectious diseases, and some of these interventions are obligatory in certain countries. Some diseases are eliminated in many countries (tuberculosis, brucellosis) or even eradicated worldwide (rinderpest). Controlling disease-transmitting vectors (e.g. insects, ticks) and breaking the cycle of gastrointestinal parasites are essential preventive measures.

Moreover, certain diseases (zoonoses) are common to animals and humans (Table 1.8). Raw milk is the main route of transmission but other animal products and contact are also involved. Keeping livestock in close proximity to concentrations of humans increases the risk of transmitting these diseases. Special care with disease prevention is therefore necessary (see also section 1.12.2). Recently, new diseases have emerged that seriously affect livestock and may even be transmitted to humans. Bovine spongiform encephalopathy (‘mad cow disease’) has received much attention because of a possible link with the life-threatening Creutzfeldt–Jakob disease of humans (Kimberlin, 1993). Although tuberculosis has been eliminated in most industrialised countries, it is still a major killer in many tropical countries and mainly transmitted by raw milk. Rift valley fever may also be transmitted by milk from animals (Konrad *et al*., 2011). Infection of humans is rare but mortality rate in those infected is high (World Health Organization, 2007).

1.8 REPRODUCTION

Physiologically, milk production is part of reproduction, because it allows nutrition of the newborn. Dams secrete milk following parturition. Therefore, regular pregnancy is an essential precondition for herd productivity in milk production systems. In addition, dams should continue milk secretion when they become pregnant again. Originally, in most mammals the hormonal effects during nursing inhibit reproductive functions and milk secretion ceases during renewed pregnancy. While in dairy breeds the reproductive process is accelerated, beef breeds and less-developed tropical breeds, Zebu and Zebu crosses in particular, are late maturing, generally do not exhibit oestrus while lactating, and cease lactating if pregnant again.

Sexual maturity is attained at different ages in the various species and breeds. Age at first parturition is economically important. Commonly dairy cattle calve for

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Table 1.8. Zoonoses.

Disease	Organism	Prevalence	Morbidity/mortality
Sleeping sickness*	*Trypanosoma rhodesiense*	Epidemics†	High
Anthrax	*Bacillus anthracis*	Low	
Bovine spongiform encephalopathy	Prion	Rare	Low
Brucellosis	*Brucella* spp.	Frequent, tropics	High
Food poisoning	*Salmonella* spp.		
Food poisoning	*Campylobacter* spp.		
Haemorrhagic colitis	*Escherichia coli* O157		
Leptospirosis	*Leptospira* spp.		
Listeriosis	*Listeria monocytogenes*		
Pasteurellosis	*Pasteurella multocida*		
Q-fever	*Coxiella burnetii*	Frequent, tropics	
Rift Valley fever	Rift Valley fever virus	Rare	Low
Tick-borne encephalitis	Tick-borne encephalitis virus		
Tuberculosis	*Mycobacterium bovis*	Frequent, tropics	High
Zoonotic diphtheria	*Corynebacterium ulcerans*		

*Human African trypanosomiasis.

†East and South Africa.

Source: based on data from National Consortium for Zoonosis Research (2011).

the first time at 24 months of age, sheep and goats at 12 months, mares at 3 years. However, unduly accelerating the maturation process by intensive feeding can have negative effects on reproductive function and longevity (Essl, 1998). Dairy cows can calve in 12-month intervals. For this to be achieved, taking into account the 9 months duration of pregnancy, cows must conceive again within 3 months after parturition. This requires rapid restoration of complete functionality of the genital tract and ovary after birth (Svennersten-Sjaunja & Olsson, 2005). Timely mating (AI in particular) is important to keep calving intervals within the desired time frame. High-producing cows have a tendency to show only weak and short oestrus symptoms. Heat detection is critical. Physical observation is time-consuming and unreliable (three times daily observation of the cows over 30 min is the recommended practice). Improvement in oestrus detection is sought with technical appliances such as registration of concentrate intake and behaviour pattern with telemetric methods, automatic detection at milking and analysis using computer models of changes in physical milk characteristics that indicate oestrus (temperature, electrical conductivity). The latter should also serve to detect early signs of udder inflammation (mastitis) (De Mol, 2000). As milk yield is at its peak at the beginning of lactation, when metabolism and all body functions are extremely challenged, malfunctions can only be avoided with the most sophisticated animal management.

In practice, the average calving interval is more than 12 months and calving rate is about 80% in most dairy herds. In general, with the strong increase in milk yield, the fertility of dairy cows has declined (Royal *et al*., 2000), for example the calving rate is declining at a rate of 1% per year in the UK. Some herdsmen find it appropriate to give high-yielding cows a rest period after three or four lactations by postponing rebreeding for several months. Also, there is some evidence that extending lactations to 18 months rather than 12-month cycles can be beneficial (Sorensen *et al*., 2008). Although hormonal treatment may have immediate effects in some cases, any long-term strategy must rely on improving nutrition and genetic disposition. While the heritability of all the traits involved with reproduction is low, it should be included in breeding programmes because of its great economic importance (Pryce *et al*., 1997).

In the tropics, reproductive rate is generally lower than in temperate zones. Late maturity and extended parturition intervals are typical (depending on breed and husbandry conditions). Energy, protein and vitamins (vitamin A in particular) are in short supply during the tropical dry season but minerals (phosphorus in particular) may be deficient all year round. Also, high ambient temperature directly reduces fertility of males and females (De Rensis & Scaramuzzi, 2003). In pastoralist herds and on ranches in semi-arid areas, calving rate is between 40 and 60%. However, even in tropical humid areas calving rate does not exceed 60–70% on average.

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The reproductive rate of goats is high. They are precocious, bringing their first kid at 1 year of age. With a pregnancy duration of 5 months, the kidding interval of goats is usually 12 months but may be extended by the farmer. Small ruminants can easily achieve yearly lambing and kidding even under tropical conditions. The annual reproductive pattern of wild ruminants is governed by the photoperiod in the temperate zone, so that parturition and rearing are adjusted to the seasonal environmental conditions. This pattern has been lost during the domestication of cattle, but has been retained in most sheep and goat breeds so that they reproduce seasonally.

1.9 REARING OF YOUNGSTOCK

Undisturbed growth during development is a precondition for healthy and productive adults. The natural process of nursing by the dam is of course the optimum. However, it is difficult to combine with milking, although this is done in low-production systems where milk in excess of intake by the calves may be milked. Typically in this situation, the nursling is separated from the dam during the night and the milk (secreted continuously over 24 hours and stored until withdrawal) is obtained by milking in the morning. In systems with high levels of milk production, the general practice is to separate calves from the dam early. As the secretion of the udder for about 5 days following parturition (colostrum) is not considered suitable for human nutrition, calves are often left to suckle during this period. However, as cows for milking must be accustomed to the stimulus of the milker, any previous experience with nursing may have a negative impact. The dam is not strongly attached to the young immediately after parturition and bonding develops only gradually. Allowing the calf to suckle and then weaning it after even a short suckling period is painful for both dam and nursling (Stěhulová *et al*., 2008). Also, after weaning the calf may not easily adjust to other methods of milk intake (bucket or nipple feeding). If separated immediately after parturition, the cow 'forgets' the young. Thus, not allowing any contact between dam and calf has proved to be the most practical procedure, although it may not be acceptable to all for animal welfare reasons (Flower & Weary, 2003).

Because of the high value of milk, and milk fat in particular, producers examined the opportunities to feed calves with cheaper products (FAO, 2011a). The industry came to their aid and milk replacers were developed based on skimmed milk powder. Today, almost universally (except in extensive operations in developing countries with low-producing cows which nurse their calves and are milked simultaneously), calves receive colostrum for 10 days and milk replacers afterwards but are bucket-fed all the time. However, in periods of excess milk production, as experienced during the past decennia in Europe, this practice does not make sense. When milk in excess of prescribed quota is paid below cost of production, it may be more economical to feed this milk to calves. Also, in biological/organic operations, milk replacers are not allowed (see section 1.2.2).

As mortality among age groups in dairy herds is highest in calves, these need particular attention. For hygienic reasons calves may be kept in close confinement in single boxes on bedded ground or on slatted floors during the first weeks of life. Strict sanitation is possible under these conditions, and infectious diseases, diarrhoea and lung disease in particular, which are a permanent menace under intensive management, can be controlled. With well-organised management and close attention, calf mortality in large herds (>500 cows) can even be lower than in smaller herds (USDA, 2007). However, in very small herds (up to 50 cows) mortality during the first 6 months of life increases with herd size (Gulliksen *et al*., 2009). Computer-controlled fully automated nursing systems for calves as well as for dairy goats in commercial herds have been adopted, for example in the USA, France and Taiwan (Earleywine *et al*., 2011).

1.10 HOUSING

Dairy animals are housed under a wide variety of conditions. In southern countries, on small-scale holdings for family milk supply they may just be tied under a tree or a simple roof. Animals that are grazing all year round will be kept in a night enclosure protected from adverse weather, predators and theft. Pastoralists may keep sheep or goats at night in their huts, tents or corrals. When milk is produced for marketing, facilities are required to keep the animals clean and to obtain milk under hygienic conditions. In advanced dairying, animals are housed in elaborate barns, including sufficient ventilation, water spray cooling and even air conditioning. There are several systems where a synthesis is sought between animal comfort, cleanliness, ease of animal handling and minimised workload.

In stanchion barns, animals are tied individually. Bedding may be provided and changed daily to be stored for later use as composted manure. Where straw is in short supply and animals stay indoors only overnight, the floor may be bare or covered with rubber mats. Faeces and urine are channelled into a reservoir to be handled as slurry. Feed is offered in a trough which may be separated by a grate for individual feeding. Animals may be milked in the stall or led to a milking parlour. Nowadays these barns are acceptable for animal welfare reasons only when animals are left out for grazing during the day, or for a limited winter period.

In loose housing, animals can move freely within a barn with continuous access to a feed trough and water supply.

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The loafing area is without bedding and will be cleaned with (a minimum volume of) water. The whole area or part of it may be fitted with slatted floors to facilitate transport of the excretions and dirt to a reservoir. Slurry is produced. Stalls with or without bedding (straw, wood chips, sawdust, sand or recycled dried manure) or rubber mats are provided where animals can rest individually. They are milked in a milking parlour. Loose housing can be combined with milking robots and electronic devices for computer-controlled self-feeding. If natural ventilation in the barn is not sufficient, mechanical ventilation with fans may be necessary, under hot and humid conditions in particular. Loose housing prevails today, although it still is not yet universally accepted (USDA, 2007).

Ewes and most goats are kept in loose housing or in elevated barns with slatted floors. This helps to control reinfestation with internal parasites in tropical countries in particular. Droppings are collected for later use as manure, which is sometimes even sold to gardeners, for example in Taiwan (Morgan, 1996).

Although manure from straw bedding is best disposed on agricultural fields, the labour requirement is high and sufficient acreage may not be available. Accumulation of manure has become a problem in dairying systems that are not linked with agriculture. Slurry handling can be mechanised but there is a danger of environmental pollution. However, slurry can be a source of profitable biogas production to produce electricity on the farm and thereby emissions of methane can be reduced.

1.11 MILKING

Milking procedures and facilities are more extensively covered in Chapter 3. Milk is formed continuously in the secretory tissue of the udder. It accumulates in the milk ducts and to a lesser extent in the cistern. In order to be available to the suckling young or dairyman, it must be moved entirely into the cistern. (Note that not all the milk present in the udder can be extracted: a certain 'residual' proportion always remains, more often with milking, depending to some extent on the quality of the milking procedure, than with suckling.) This movement of milk into the cistern is facilitated by muscle-like cells surrounding the milk-secreting alveoli. They contract and squeeze out the milk under the effect of a hormone, oxytocin. This is released from the pituitary gland (hypophysis) by neural stimuli connected with nursing, i.e. tactile stimuli of the udder and teats but also behavioural stimuli connected with fostering (Svennersten-Sjaunja & Olsson, 2005). The decisive step in the evolution of domestic dairy animals was the ability to trigger this so-called milk let-down reflex purely by manipulating the udder at milking, without the presence of the calf. This is most marked in European dairy cattle and goats due to the success of genetic selection. In the extreme, milk will squirt from the udder (especially with weak sphincter muscles, which close the opening of the teat) even when the cow (or goat) is anticipating the act of milking, without any mechanical stimulation. In all other breeds and species used for milk production, the presence of the nursling is necessary to initiate milk extraction.

Milking can be by hand, which is sufficient with limited milk yield. The milk is obtained by squeezing the teat, the necessary pressure being determined by the tension of the teat sphincter. This pressure can be rather high so that milking is hard labour. Therefore, and to save labour time, milking with machines was developed and today is practised almost exclusively in developed countries. Usually, cows are milked twice daily, but in high-producing herds thrice daily milking is profitable and common today.

At the beginning of the age of technical developments, the milker would take the milking cluster to the individual cows. Nowadays the cows are made to walk by themselves into a milking parlour. Milk is either collected into a pail or fed into a pipe system that channels it directly into a tank where it is cooled prior to being collected by the dairy factory. Further improvements in milking devices were brought about by the introduction of milking robots. Cows fitted with electronic identification systems enter the milking robot stall when they feel like milking and when the system indicates that it is time for milking and concentrate feeding. The robot identifies the cow, cleans the udder, checks for quality milk from each quarter, attaches the cluster, supervises milk flow, detaches the teat cups when milk flow ends, disinfects the teat end, and finally makes the cow leave. The system provides for more flexibility in work organisation, although the time saved for milking is needed to maintain and clean the robot system (Rotz *et al*., 2003).

Hygiene in the milking process is important for both milk quality and udder health. It is maintained by proper cleansing of the udder and the whole milking line, including the application of disinfectants and by testing the milk physically and chemically for the absence of mastitic conditions prior to attaching the milking machine teat clusters.

1.12 MILK MARKETING

A well-organised marketing system serves the interest of both consumers (ensuring supply of safe, quality milk) and producers (ensuring market outlet at appropriate prices). In general, marketing problems are due to (FAO, 2000):

- the short shelf-life of the perishable milk produced daily;
- the cost of collection and transport;
- the varying volume produced during the course of the year;

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- a risk of milk adulteration by skimming or watering in an uncontrolled collection and marketing system;
- the need for processing before its end use;
- the possibility of spreading zoonoses (e.g. brucellosis, tuberculosis).

In modern dairying systems, common practice today is that milk is channelled directly from the milking parlour into a cooling tank. It is stored at low temperature until shipped to the dairy factory, daily or even every second day. In the creamery, milk is subjected to heat treatment as soon as possible in order to inhibit (or at least delay) microbial action (see Chapter 14). Dairy factories have the potential to pollute water: while poor management results in waste loads of 3 kg BOD (biological oxygen demand, a measure of water pollution) per ton of milk, it can be reduced by good management practices to 1 kg (de Haan *et al*., 1997).

1.12.1 Marketing by smallholders

As has been pointed out, milk is an important source of income for smallholders in developing countries. In subsistence farming the marketing potential of dairy products can only be tapped directly by farmers situated close to the markets and who can themselves deliver the milk. However, the intake capacity of local rural markets is limited. While large farms can market milk or cheese directly in cities, most of the market-distant smallholders depend on organisations that purchase and collect the milk. Middlemen provide this service and in many developing countries still today up to 80% of milk is supplied to urban consumers via this so-called informal market, sometimes at half the 'official' price. However, this activity has limitations and deficiencies. Adulteration of milk (skimming and watering mainly), especially during lean periods, is a common problem. Middlemen often pass on an inadequate part of their profit to the producer and cannot purchase, or purchase only at very low prices, seasonal surplus milk. On the other hand, they often extend advance payment and act as a liaison to the marketplace. However, their intake capacity and trading area is limited to a certain perimeter around cities. Even the dairy factories prefer to collect milk close to the cities because milk collection over long distances is expensive due to road conditions in outlying areas in countries with insufficient infrastructure in particular. These dairies are unable to compete with the middlemen because of high fixed costs and expenses incurred by following government marketing regulations. In order to enable producers in outlying areas to benefit from any marketing opportunities, a well-organised milk collection system is required. This is in the interest of both the small farmers and the dairy factories who need sufficient volume to operate economically. If milk collection is expensive, dairy factories in some countries prefer to reconstitute milk with skimmed milk powder and butter oil (provided there are no import restrictions and the price is right; sometimes expensive butter oil is replaced with vegetable oil to produce 'filled milk'). In doing so, they deprive the small milk producer of the benefit of marketing their milk. Government regulation may be necessary to prevent this (see the example of Operation Flood in section 1.12.3).

1.12.2 Milk collection

In the past, farmers would bring their small quantities to a collection centre, or trucks collected milk cans placed on the collection route. Often by the same route, skimmed milk (and whey) was brought back to the farmer who used it as animal feed (for pigs mainly). Today, dairy factories collect cooled milk with road tankers at the farm gate. In order to expand the collection radius and to lower costs, multi-tier milk collecting systems are employed in countries with deficient infrastructure. The small quantities from individual producers are collected at collection points from where it is brought to collection centres from where it is shipped to the dairy factory. Where infrastructure permits, one of the stages may be skipped (e.g. producers bringing milk directly to the collection centre or even to the creamery). With the multi-tier arrangement, the difficult and most expensive part of transport (off road) is covered by producers on foot, on donkey or horse back, on bicycles or small vehicles, and the following stage on poor dirt roads by pick-ups or small trucks. Only at the final stage do road tankers ship the milk to the dairy factory. Collection centres need a minimum amount of milk to operate economically. They are preferably located on a road (hard surface) to reduce the requirements for expensive transport. On the other hand, they should be close enough to the producer to keep distances short.

At the collection centre the milk is generally cooled (collection and cooling centre). If there is no option for cooling, only the morning milk may be collected and shipped to the dairy factory the same morning. Cooled milk is shipped to the dairy daily or every second day. There it will be pasteurised, processed and delivered to the retail trade. Frequently, collection centres operate as sales points for local consumption. Additionally, they may function as small social and commercial centres. Large dairy farms in areas with insufficient road structure ship their milk to the creamery in cans or with road tankers.

Originally, milk was used sweet fresh or boiled. Worldwide, since the beginning of modern dairy development, consumers and health authorities have been concerned about the possibility of disease transmission through milk (see section 1.7). Sanitation via heat treatment was introduced universally in the second half of the

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nineteenth century. However, this puts an economic burden on small milk producers in particular. As consumers traditionally are used to boiling milk prior to consumption, the prior heat treatment seems not to be necessary in all situations. For example, Kenya recently changed dairy policies, legalising the sale of unpasteurised milk by the informal trade with economic benefits to producers (Owango *et al*., 1998; ILRI, 2008). In Zambia, milk is used mainly sour (coagulated) as additive to certain dishes. Dairy farmers let their milk turn sour and deliver it directly to retailers who sell it until noon (personal observation).

Because milk output varies during the course of the year, producers very early developed procedures to conserve milk. The simplest form is sour milk, which is prepared in various forms including some subject to alcoholic fermentation. Fat can be separated and conserved as butter or transformed into ghee. Cheese requires fairly sophisticated technology: soft cheese for short shelf-life (except for Feta cheese in brine, which gives it a long shelf-life) or hard cheese for longer preservation. In the dry tropics, milk is also dried for conservation.

1.12.3 Producer organisations

In order to reduce dependency on the milk trade, the implementation of producers' associations (dairy cooperatives) for milk collection, processing and sale is essential in rural areas. It was key to dairy development in Europe at the onset of the cooperative movement in the nineteenth century. Even today most dairy farmers in the USA market their milk through cooperatives (US Government Accountability Office, 2004) and about 30% in Germany (Raiffeisen, 2012). In order to be competitive in the market, many cooperatives have developed into large dairy companies. Dairy cooperatives run a milk collection system and may sell milk to an industrial plant or run their own milk factories, taking care of processing and sales of milk. In addition, cooperatives provide services to member farmers (milk recording, training and advice, input procurement, insemination and veterinary service, credit).

Of major importance are the dairy cooperatives in developing countries because they open marketing opportunities to small farmers. An outstanding example in the recent past is Operation Flood, a large-scale project by the Indian National Dairy Development Board (NDDB, 2010) started in 1970 with financial support by the European Union, the World Food Programme and a World Bank loan. It is considered one of the most successful projects of development assistance in the era after the Second World War. Also called the 'White Revolution', alluding to the 'Green Revolution', it endeavoured to establish a country-wide supply grid with milk production based on dairy cooperatives. They started by organising milk collection, taking even the smallest quantities of milk. In order to make the expensive system operate, the dairy factories initially, while sufficient milk volume was not yet collected, reconstituted milk with skimmed milk powder and butter oil. NDDB was granted exclusive licence to import these commodities, which for the rest was prohibited. Cooperatives formed unions who ran dairy factories and feed mills. Milk was paid directly on delivery and receipts rapidly caused improvement of farmers' living conditions. The criticism that sales would deprive the family of the milk needed for family feeding, infants in particular, was invalidated by proof that with the returns from milk sales it was possible to purchase six times more nutrients in other food (July 1979; see also section 1.2.5). A system for quality control, payment of producers, conservation, transport, milk processing and sale was established. Cooperatives developed into strong self-help organisations assisting with procurement of inputs (feed, fertiliser), credit, AI and veterinary service, extension, training and breed improvement. Economic success eventually allowed the cooperatives to extend their activities to infrastructure improvement and schooling. The Indian model was successfully copied in other countries.

1.13 ECONOMICS OF MILK PRODUCTION

The most important factor in the economics of intensive milk production is milk yield. Lifetime income is 17.4% higher with cows selected for high milk yield than with their average herd mates. Although the cost of production has also increased, the net result of high yield is positive (Dunklee *et al*., 1994a). It is often suspected that very high milk yield is genetically associated with more health problems and reproductive disorders and much research, both statistical and experimental, has been devoted to this question. Results are inconsistent, supporting the expectations of sceptics but also of those convinced about the benefits of breeding for high milk yield (Shanks *et al*., 1978; Fourichon *et al*., 2001).

The cost of producing milk depends on various cost factors, foremost feed, cow replacements (see also section 1.13.2), labour, healthcare and investments. As these factors vary greatly between countries, overall cost differs accordingly. A cost comparison for the major milk producing countries of the world is shown in Table 1.9. In the EU, higher costs are due to unfavourable weather conditions requiring housing of cows in wintertime, high labour costs, small farm and herd size, and the milk quota system (Deblitz *et al*., 1998a, b).

In industrialised countries there is a general tendency to produce more milk than the market can absorb, with

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Table 1.9. Cost of milk production worldwide.

	US$/100 kg milk
EU farms with around 30 cows	50
EU farms with 60–75 cows	38
USA, Central Europe, Brazil and South Africa	25–30
Argentina, Uruguay, Australia and New Zealand	20

Source: based on data from Deblitz *et al*. (1998a, b).

Table 1.10. Returns from animal production.

	Returns on total energy (E) and protein (P) inputs		Returns on human-edible energy and protein inputs	
	E (%)	P (%)	E (%)	P (%)
Milk	23	29	101	181
Beef	5	5	57	109
Swine	23	38	58	86
Poultry	15	30	31	75

Inputs were calculated as digestible energy and protein and include costs of maintaining breeding.

Source: based on data from Bywater & Baldwin (1980).

resulting pressure on producer price. Very small farm properties prevail in many European countries as a consequence of inheritance laws, which split the bequest equally among all the heirs. Their acreage does not provide sufficient family support except by intensive systems of production. As dairying is one of their mainstays, it is often subsidised. In Europe (and similarly in North America) with varying success attempts have been made to maintain producer prices by several market intervention measures: price support through intervention buying, import tariffs and export subsidies, and milk quotas to limit production levels (Commission of the European Communities, 2002; European Union, 2007a). However, since at least 2007, world market prices for dairy products have risen sharply (doubling or even tripling in some years), so that the phasing out of any subsidies was considered in 2011 and EU quotas will be abandoned by 2015 (European Commission, 2010).

In developing countries, unwanted effects result from promotional measures of governments, for example the lack of standards for large-scale farms or even their financial support. Policies prevail to provide milk at moderate prices to the urban population even to the detriment of rural producers (Morgan, 2009).

1.13.1 Productivity

The animal breeder may be interested primarily in a cow's milk yield. Economic considerations require the determination of productivity. This is commonly expressed as production related to input factors. Depending on the individual situation, one may consider feed productivity, land productivity, labour productivity or capital productivity. However, income over feed cost is a practical indicator of economic performance. Where human food is in short supply, the conversion of nutrients may be decisive for justifying animal production and that of milk in particular. As can be seen in Table 1.10, the utilisation of nutrients that can be consumed directly by humans is best through milk production. Returns can exceed 100% because microorganisms of the digestive system synthesise protein and are digested by the ruminant host. As pointed out, milk production can be an important source of family income in developing countries. There, the return on labour may be most important as long as alternative sources of employment are not available.

1.13.2 Longevity and lifetime production

In most modern intensively managed dairy herds, most cows are removed at about 6 years of age having undergone no more than three to four lactations, before reaching the peak of their productive potential (Essl, 1998). Over the recent past, longevity has decreased in most European dairy cattle populations because selection was based on milk yield, mainly assessed early in life. Reasons for this are ill health (metabolic and udder diseases), foot problems and reproductive failure (Weigel, 2010). However, longevity is the most important factor influencing economics, because the cost of replacement is an important part of the overall cost. It was the second cost factor behind feed cost on California dairy farms in 2002 (US Government Accountability Office, 2004). A recent study in Germany revealed that cost per kilogram milk was as follows: labour €0.10, roughage €0.07, concentrates €0.07, replacements €0.06, respectively (Rindfleisch & Heber, 2009). Lifetime production combines yield with longevity and is probably the best indicator of sustainable production by healthy reproductive cows and thus is the best indicator of animal welfare. Unfortunately, the heritability of this trait is low and therefore it cannot easily be improved by selective breeding (Vukasinovic *et al*., 2001). However, a number of dairy herd associations have impressive numbers of records of 100 000 kg lifetime production that requires 10 lactations of 10 000 kg each, permanent health and undisturbed sequence of calving, which can only be achieved by

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well-managed and well-bred cattle (German Genetics International, 2010). Consequently, today, so-called secondary or functional characteristics (in addition to milk yield), which are preconditions for longevity and high lifetime production, are becoming increasingly important and these include reproductive function, general health, sound feet, healthy and easily milked udders, and eager feeders (Groen *et al*., 1997; Gay *et al*., 2011). However, careful skilled management that provides a healthy comfortable environment is also essential.

1.14 CRITICISM OF MILK PRODUCTION

1.14.1 Resource use

Recently, animal husbandry has been increasingly criticised for the low efficiency of resource use (mainly land and water) compared with the production of vegetable food. One consequence is increasing reluctance to assist livestock development in countries that have difficulties in providing sufficient food for their population (Steinfeld *et al*., 2006). While the focus of this criticism is mainly on beef (and other meat) production, to some extent it also includes dairying but to a much less degree. Nevertheless, this critique needs to be qualified. Worldwide about 70% of agricultural land is pasture, varying greatly between countries (from over 90% to less than 5%) (FAO, 2011b), much of which is used, at least partly, for milk production. In smallholder dairy systems much use is made of agricultural by-products. In addition, fodder is grown as a winter crop between grains. These feed resources cannot be used better than for dairying. However, fodder is certainly grown as the sole crop where it competes with crops directly usable as human food. Worldwide, this area comprises about 14% of agricultural land but with great differences between countries, for example in India it is only about 4%.

In response to rising demand and better marketing opportunities, specialised intensive dairy operations are growing. They improve animal efficiency by adding grains to the feed. How much grain is fed to dairy animals is difficult to estimate but certainly a balance should be found between adequate supply of dairy products and optimal resource use, considering that the resources needed to produce animal products generally exceed those to produce agricultural food commodities for human consumption (cereals, sugar and oil plants, see also section 1.3), although milk production compares quite favourably with other lines of animal production (see Table 1.10).

1.14.2 Impact on the environment

Presently there is growing public concern about the environmental aspects of livestock farming practices including dairying (Place & Mitloehner, 2009; Croney & Anthony, 2011). As livestock farming expands, there is a tendency in the dry tropics for over-grazing and in sub-humid and humid zones for expansion into sites prone to erosion as well as invasion of rainforests (Steinfeld *et al*., 2006). Continued over-grazing may lead to desertification, while grazing of steep sites in sub-humid and humid zones may cause erosion. Wildlife may be endangered by ousting of game and control of possible predators to protect livestock.

Livestock produce greenhouse gases (CO_2, nitrous oxide and methane). It is estimated that livestock cause 18% of greenhouse gas emission (in CO_2 equivalents) from human-related activities (Steinfeld *et al*., 2006) and the dairy sector including emissions from processing and transportation contributes 2.7%. It would increase to 4% if meat production associated with dairying was included. About 52% of it is methane (Gerber *et al*., 2010). Globally, livestock are the largest source of methane, which is produced when ruminants digest plant fibre (cellulose). Greenhouse gas emissions per kilogram of milk are estimated to be high in arid grassland systems (low milk yield and low feed digestibility), but low in temperate humid zones (mostly industrialised countries, high-producing cows). Emissions can be reduced mainly by efficient feed use, i.e. feeding highly digestible rations rather than high roughage rations (Hegarty *et al*., 2007; Paul *et al*., 2009). It has been estimated that feed supplementation can reduce methane emissions of dairy animals by 25% while increasing milk production by 35% (de Haan *et al*., 1997). Dairy production in the USA in 2007 caused less damage to the environment than in 1944 (between 10 and 56% of the 1944 values depending on the parameter considered) (Capper *et al*., 2009) as a result of improved management and technology as well as reduced resource use (feed, cropland, energy, water), waste output (manure, N, P), and greenhouse gas emissions. This would contradict the allegation of poor environmental stewardship of intensive dairying. Methane is also emitted from excreta, but properly handled manure is not environmentally harmful when applied on fields and pastures. Yet excessive numbers of animals on small acreages, as well as slurry from large operations, can pose problems and pollute groundwater. An EU directive stipulates the procedures to avoid pollution (Council of the European Communities, 1991).

Most extensive peasant livestock systems are climate friendly (Paul *et al*., 2009).They can be extremely efficient at enriching biodiversity and in sequestering greenhouse gases. The advantage of extensive livestock is revealed only if looked on as a system in which mainly land and agricultural resources are used that cannot be used directly as human food. There is also growing public concern about animal welfare (Keyserlingk *et al*., 2009). One of the main

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issues is deprivation of dam and suckling young of the benefits of dam care (see section 1.9).

1.15 DAIRY DEVELOPMENT

Demand for milk increases with growth of the urban population. Furthermore, as income grows, people tend to increase consumption of higher priced livestock food. Consequently, milk production is enhanced. As can be seen in Table 1.11, worldwide total milk production over the last decade increased by 28% but per-head availability lagged behind. In food-deficit countries, it is less than one-third that in Europe. Milk is a staple food for many livestock owners who produce for self-supply and not for the market, such as smallholder farmers or pastoralists. Milk easily supplies protein and energy, which are both deficient in malnutrition in most cases. In developing countries, a sufficient supply of milk and dairy products to the needy (infants, pregnant women, elderly, diseased) should be ensured without unduly reducing the production of basic vegetable food (Pimentel, 2009). Ideally, most of the milk should come from small producers (Preston & Leng, 1987). In integrated farming systems, livestock barely displace cropping while animals fed mainly on pasture and agricultural by-products have positive effects beyond food production by contributing to soil conservation and fertility (manure) and by providing draught power (Nellemann *et al*., 2009).

The International Food Policy Research Institute (IFPRI) estimates the price elasticity of dairy products in low-income countries at –0.7 as compared with high-income countries at –0.3 (Braun, 2007), while indices of income elasticity of demand for milk are high in developing countries, estimated at between 1.43 and 1.26 (Delgado *et al*., 1999). Thus, the poor can barely afford expensive dairy products and this is aggravated by rising commodity prices. However, if governments try to make dairy products available to the urban population by keeping prices low, they thwart dairy development and furthermore curtail possibilities for rural producers to take advantage of the income-generating opportunities of milk production. Remunerative producer milk prices can act to transfer purchasing power from city to rural areas, whereas milk supply to the needy may be secured by direct subsidies.

Promotion of animal husbandry, dairying in particular, can trigger the improvement of living conditions of the rural family, with repercussions on crop yields directly, or indirectly via the opportunity to utilise revenues in cash for the purchase of inputs. Moreover, as dairying is often the domain of women, promotion of milk production can contribute to their well-being (FAO, 2004; see also Operation Flood). Furthermore, there is a sociocultural aspect in societies where dairy animals, cows specifically, play a role beyond their economic importance (sacred cows in India, camels with pastoralists).

Development programmes mainly aim at the following (Ndambi *et al*., 2007):

- policy formulation in support of smallholders;
- price control and appropriate administration of food aid imports;
- support of collection, processing and marketing of milk and dairy products preferably through producer associations;
- genetic improvement of dairy animals;
- improving veterinary services;
- training.

International organisations such as the World Bank, IFPRI, Food and Agriculture Organisation of the United Nations

Table 1.11. Changes in world milk production from 1997 to 2007, total and percentage change.

	Million tonnes			kg/capita/year		
	1997	**2007**	**%**	**1997**	**2007**	**%**
Low-income food-deficit countries	86.4	135.9	57.3	24	32	35
World	257.8	329.8	27.9	44	50	13
Africa	17.1	26.9	57.1	23	28	24
Asia	94.2	139.5	48.1	27	35	31
Europe	68.8	67.8	–1.4	95	93	–2
India*	42.2	47.1	11.7	43	41	–5

*Data are misleading as a plateau was reached in 1985; growth between 1975 and 1985 more than doubled to 31 million tonnes.

Source: based on data from FAO (2011b).

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(FAO) and the International Livestock Research Institute (ILRI) envisage a future 'Livestock Revolution' with the following characteristics (Delgado *et al*., 1999):

- rapid worldwide increases in consumption and production of livestock products;
- a major increase in the share of developing countries in total livestock production and consumption;
- an ongoing change of livestock production from a multipurpose activity with mostly non-tradable output to food market production;
- an increased use of meat and milk for grain in the human diet;
- a rapid rise in the use of cereal-based (animal) feeds;
- greater stress put on grazing resources along with more land-intensive production closer to cities;
- the emergence of rapid technological change in livestock production and processing in industrial systems.

These prospects certainly apply to dairying. Assisting developing countries with dairy development will improve the supply of milk and dairy products to consumers and at the same time, if well guided, improve the situation of small farmers.

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2
Mammary Secretion and Lactation

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2.1 INTRODUCTION

Mammary secretion, called "milk," is the most complete food in nature. It is a source of essential nutrients not only for the neonate of any mammalian species, but also for growth of children and nourishment of adult humans. It is produced through the process of lactation of mammalian species.

The mammary gland, like sebaceous and sweat glands, is a cutaneous gland (Jacobson, 1975). In the more advanced mammals such as farm animals, the mammary gland is histologically a compound tubuloalveolar type, which originates from the ectoderm (Jacobson, 1975). Even though the mammary gland is characteristic of and basically similar in all mammals, there is wide variation among species in the relative amounts of the components in the secretion (Schmidt, 1971; Jacobson, 1975).

Lactation and mammary secretion take place in a series of integral stages in the mammary gland. These include mammary cell growth (mammogenesis), pregnancy, milk ejection (secretion), maintenance of lactation, mammary cell regression (involution), and dry period. Lactation is governed by specific physiological, endocrinological, and biochemical functions that are closely linked to reproductive functions of mammals. The initiation of the milk secretion process is under the influence of the hormone oxytocin, which is released via neurotransmission of nerve impulses from the mammary gland to the spinal cord, the hypothalamus and posterior pituitary gland of the brain. Milk secretion is highly regulated by endogenous biological cycles, environmental factors and management strategies at the farm level for dairy animals (Schmidt, 1971; Hafez, 1974).

Humans have domesticated some mammals, and over the years have selected and bred them to produce volumes of milk far in excess of that needed to nourish the young. This excess is the basis of the dairy industry, one of the most important agricultural enterprises. In order to have a successful dairy production, the lactating animals must be under constant optimum management conditions. The yield and composition of milk is influenced by many physiological, environmental, management, and individual animal factors. Factors affecting increases in milk yield of cows include increased body weight, advancing age, increased plane of nutrition, fall and winter calving, moderate or cool environmental temperatures, and good body condition at calving (McDaniel & Legates, 1965; Schmidt, 1971). Factors which tend to decrease the yield of milk include advancing lactation, advanced stage of gestation, short dry period, spring and summer calving, high environmental temperatures and humidity, diseases that affect the udder or feed intake of the cow, and a decreased plane of nutrition (Schmidt, 1971; Watters *et al*., 2008).

The purpose of this chapter is to delineate and review all aspects of mammary secretion and lactation related to milk production for human consumption and nutrition. It focuses on mammary gland development, milk secretion, maintenance of lactation, and mammary involution which are related to milk production and its quality.

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2.2 ORIGIN AND ANATOMY OF MAMMARY GLANDS

2.2.1 Types of mammalian species and mammary glands

Mammals include a variety of species, ranging from the monotremes, phylogenetically the most primitive, to humans, who have the most complex nervous system. The mammalian class includes 3500 existing species and 1000 living genera (Schmidt, 1971). This class is divided into two subclasses, the Prototheria, the egg-laying mammals, and Theria, mammals that bear live young.

Monotremata is the only order under the Prototheria subclass, and the monotremes are egg-laying mammals that have no placenta for the development of the young (Schmidt, 1971). One species of the Monotremata order is the porcupine anteater, which develops a pouch on the surface of the abdomen and the egg is transferred to the pouch immediately after ovulation. After the egg is hatched, the young live on the milk secreted in the pouch by the dam.

The subclass Theria has two infraclasses: (i) the Metatheria, pouched animals, and (ii) the Eutheria, the placental animals (Smith, 1959; Schmidt, 1971). Marsupialia, a single order, is classified under the Metatheria. The marsupials are pouch-bearing animals that give birth to live young in a very immature state, which then develop in a primitive type of placenta or pouch with nipples providing milk. The order of marsupials includes the kangaroo and the opossum, which has as many as 20 nipples in the gland pouch (Schmidt, 1971).

The existing orders of mammals belong to the infraclass Eutheria, which have highly developed placentas and give birth to live young. These animals have also highly developed mammary glands and teats, but their sizes and shapes are different between species. The mammary gland is composed of a teat, a duct system, and lobes and lobules of secretory tissue drained by the duct system mostly into cisterns of varying sizes (Schmidt, 1971; Jacobson, 1975). Among the many classes of mammals, it is important to delineate the anatomy of mammary glands of those dairy species most commonly utilized for commercial milk production for human consumption.

2.2.2 Anatomy of mammary glands of domestic animals

The mammary glands of cows, sheep, goats, camels, and horses are located in the inguinal region. Cows have four functional glands and teats, whereas sheep and goats have two separate glands, and each teat has one streak canal to drain milk from the udder. The udder of cows is composed of two halves, each of which has two separate quarters, and each quarter has a separate teat. Each quarter is separated from the others by connective tissue and has a separate milk-collecting system (Fig. 2.1). The medial suspensory ligament arises from the abdominal wall and is attached to the medial flat surfaces of the two halves of the udder to form a septum between them. The medial suspensory tissue possesses great tensile strength (Schmidt, 1971; Jacobson, 1975). It is located over the center of gravity of the udder to give a nearly perfectly balanced suspension of the udder (Fig. 2.2).

The secretory tissue is made up of alveoli. A number of alveoli are joined together by a common duct and are surrounded by connective tissue to form a lobule. Many lobules are again surrounded by connective tissue to form lobes (Fig. 2.3). The milk is synthesized and secreted from the alveoli cells into small milk ducts, from small milk ducts to large milk ducts, then drained into the gland cistern, then through the cricoid fold into the teat cistern before being ejected out from the streak canal and meatus, which is closed by a sphincter muscle (Fig. 2.3). The larger ducts and the gland and teat cisterns are lined with a basal layer of cuboidal cells and upper layers of columnar cells.

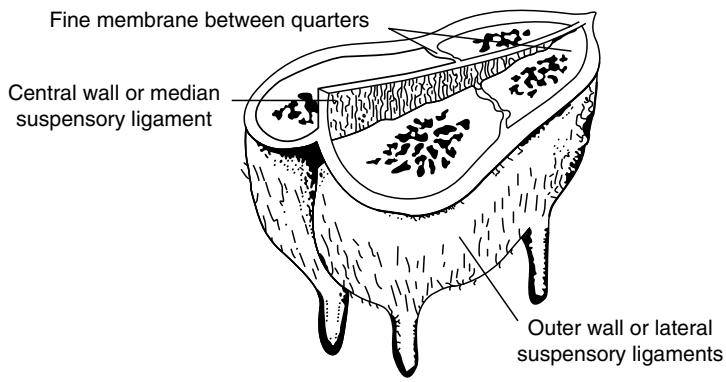


Figure 2.1. Structure of cow udder.

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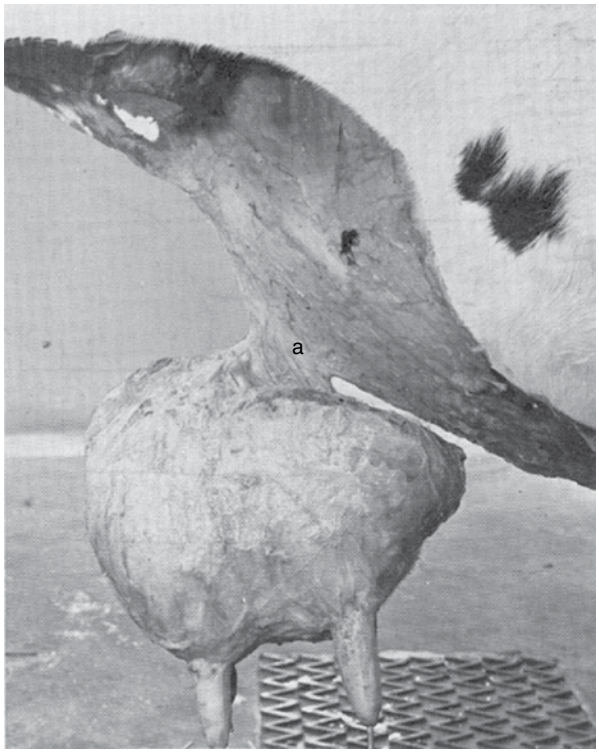


Figure 2.2. Medial suspensory ligament of cow udder. Medial suspensory ligament (a) of the udder provides nearly perfect balanced suspension of the udder. Adapted from Schmidt (1971).

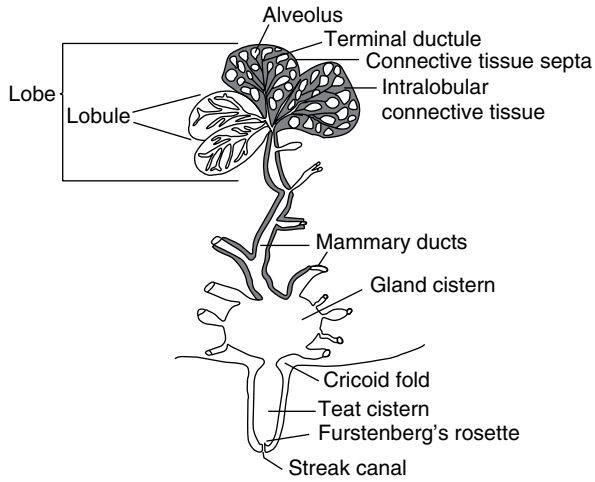


Figure 2.3. The internal organization of a mammary gland: duct and lobulo-alveolar systems. The udder is divided into four separate quarters, each independent in its milk-producing function. Adapted from Constantinescu & Constantinescu (2010), with permission of John Wiley & Sons.

Within the mammary gland there is a transition from the multilayer epithelium lining the teat meatus to the single-layer epithelium lining the lumina of the alveoli. The material lining the teat canal is keratin (Schmidt, 1971). Keratin plays an important role in preventing the entrance of mastitis-causing bacteria. The layers beneath the keratin in the teat meatus are the same as those of the epidermis. The muscle components of the bovine teat end consist of two types. Inner longitudinal muscles are found under the epithelium of the papillary eminences. The sphincter is kept under constant tension from nerve impulses from the sympathetic nervous system (Jacobson, 1975). The streak canals of sheep and goats are lined by a multilayer pavement epithelium, which rests upon a membrana propria. Longitudinal muscle bundles also surround the streak canal and are in turn surrounded by circular muscle layers. The teats of sheep differ from those of cows and goats by a slight development of the smooth muscles and a large amount of elastic connective tissue (Schmidt, 1971).

2.3 MAMMOGENESIS AND MAMMARY GLAND GROWTH

Mammary gland growth in the female takes place during five distinct phases of development: prenatal, prepubertal, postpubertal, pregnancy, and early lactation (Schmidt, 1971). Mammary glands of males in most species have similar structures to those of the female gland. However, male glands do not manifest as much growth as female glands.

The mammary gland is derived from the ectoderm and mesoderm layers (Jacobson, 1975). The general sequence of developmental changes is similar for most mammals. The mammary band, the first anlage of the mammary gland, is a broad band of ectodermal cells running on either side of the trunk from the upper limb to the lower limb. This becomes further differentiated to form the mammary line, a narrow ridge of slightly taller ectodermal cells resting on a strip of condensed mesenchymal cells from the mesoderm (Turner, 1952; Kon & Cowie, 1961). The mammary lines begin to shorten and the ectodermal cells begin to divide and grow into the mesenchymal cell layer, and form a spherical structure. In cows, there are two mammary buds in the inguinal region on each side of the mammary band that ultimately will give rise to the fore and rear quarters. The mammary bud formation is a critical stage in mammary development. The ectodermal layers sink into

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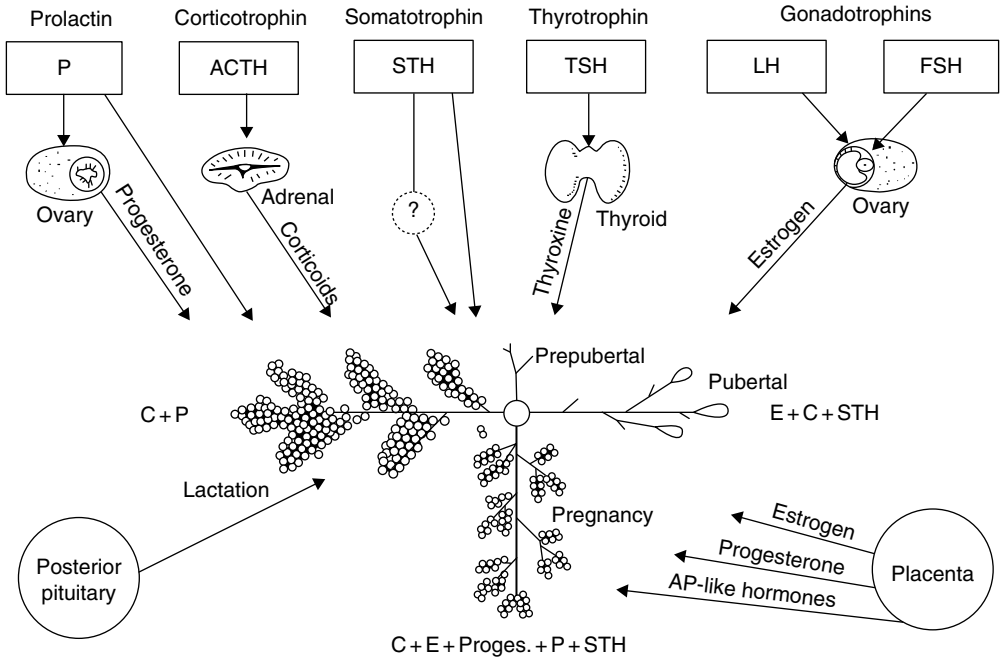


Figure 2.4. A simplified diagram showing the action of hormones on mammary growth and lactation. Upper diagram, rudimentary gland; lower diagrams, prolactational gland of pregnancy; right side, prepubertal to pubertal gland; left side, lactating gland. Adapted from Lyons *et al.* (1958).

the mesenchyme forming dimples on the embryo's surface. The mammary bud stage marks the beginning of differentiation patterns which distinguish various species. The bud stage also marks female and male glands that can be distinguished by sexual steroid action (Kon & Cowie, 1961).

An invagination of the mammary bud cells occurs in the mesenchyme, which develops into the primary sprout, and then gives rise to the gland cistern. The secondary sprouts will form the major ducts leading to the major lobes of the gland. The process of forming a lumen in the solid core of epithelial cells in the primary and secondary sprouts is called canalization, which results in forming the gland cistern first. Canalization proceeds back toward the surface, forming the teat cistern and finally the streak canal. Development of udder shape, including fat pad, begins at about 2–3 months of fetal age in the bovine (Williams & Turner, 1961; Johnsson *et al*., 1986; Berry *et al*., 2003a).

After birth, the mammary gland has an isometrical development before beginning another allometrical growth period just before puberty, i.e., at the age of 2–3 months in heifers (Purup *et al*., 1993; Berry *et al*., 2003b) or 1–2 months in young goats (Yart *et al*., 2012). In these species, mammary parenchyma differentiates into ducts that spread into the surrounding fat pads when the positive allometrical growth arrives. Secondly, lobulo-alveolar structures develop at the ducts' distal extremity. All these mechanisms of growth and differentiation are controlled by pituitary hormones (prolactin and growth hormone) and ovarian steroids (Fig. 2.4).

At the prepubertal development stage, mammary development is generally confined to growth of those parts of the mammary gland that are not clearly defined at birth, such as the sphincter around the teat meatus and smooth muscle fibers (Schmidt, 1971). This growth is not considered under hormonal control, whereas the growth occurring after puberty is attributed almost entirely to hormonal influences. In essence, the bulk of growth takes place during pregnancy and then regresses after the peak of lactation. This cycle of mammary cell growth and regression repeats itself with each pregnancy and lactation period (Fig. 2.4).

Wallace (1953) was the first to describe ovarian steroid effects on mammary gland development in ruminants at puberty. In heifers subjected to ovariectomy before puberty, mammogenesis was dramatically affected and only restored when the animal was supplemented with estradiol. Purup *et al*. (1993) also verified that in ovariectomized prepubertal heifers, parenchyma had a DNA concentration five times lower than in control intact heifers. The regulation by steroids of mammary parenchyma development

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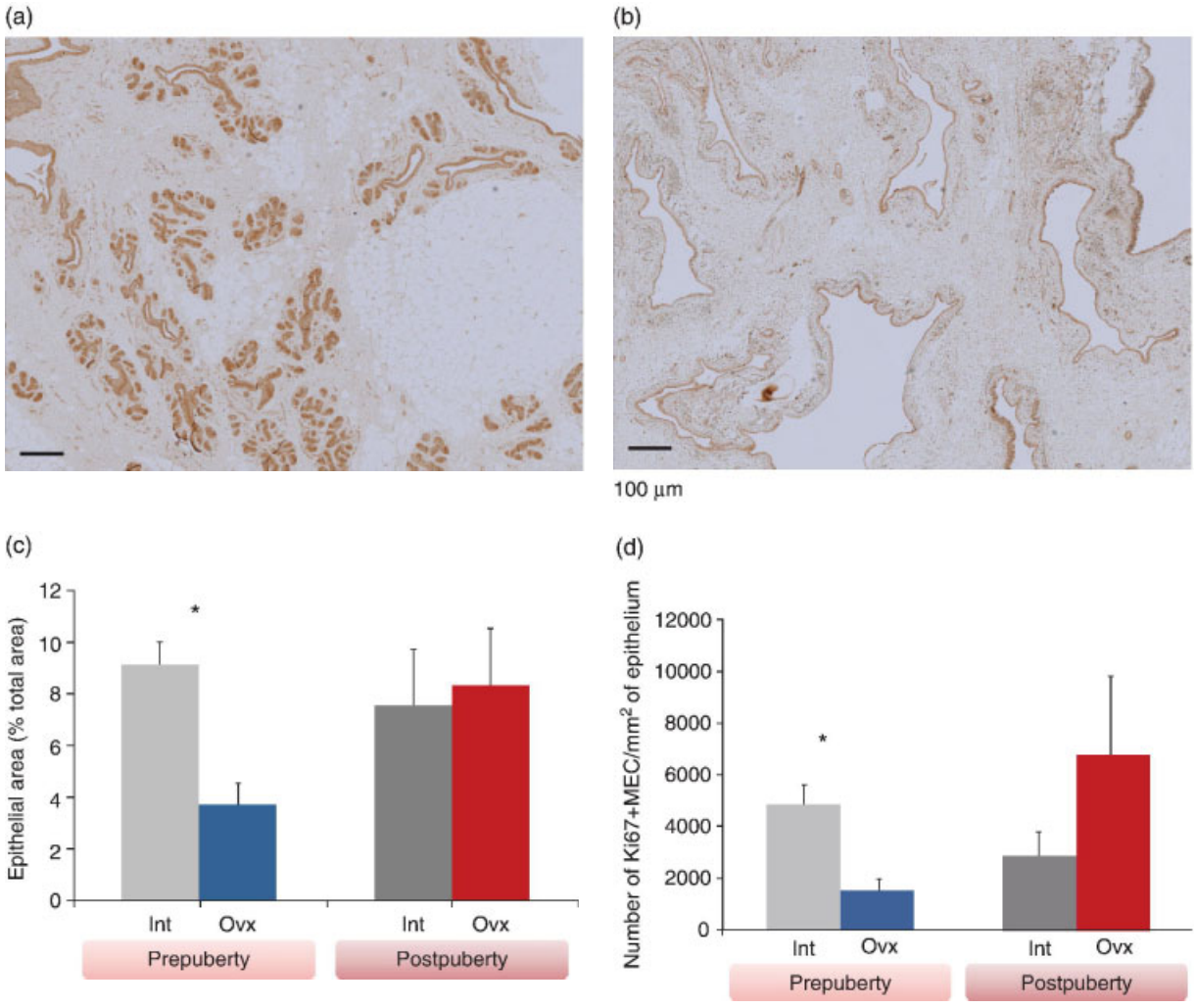


Figure 2.5. Effects of ovarian secretions on mammary gland development in young goats at puberty. Histological section of mammary gland obtained at 9 months of age from control intact young goats (a) and from young goats ovariectomized at 1 month of age (b) (bar = 100 µm). Ovariectomy before puberty affects mammary epithelium development (c) and proliferation of mammary epithelial cells (MEC) (d), but has no effect on these two parameters after puberty. * $P < 0.05$. Int, intact control young goats; Ovx, ovariectomized young goats. For a color version of this figure, see Plate 2.1.

mainly affects epithelial cell proliferation (Fig. 2.5 and see Plate 2.1). Tritiated thymidine incorporation into epithelial cell nuclei is dramatically increased (46 times) 96 hours after estradiol injection. This effect is not observed after progesterone injection (Woodward *et al.*, 1993). More recently, it was demonstrated that cell proliferation in the mammary gland of ovariectomized 2.5-month-old heifers is 10 times inferior to control heifers (Berry *et al.*, 2003b), leading to an 85–90% reduction in mammary parenchyma development in 9-month-old heifers (Purup *et al.*, 1995).

2.4 MILK EJECTION (LACTOGENESIS) AND SECRETION

Milk removal is the process in which the milk produced in the mammary gland is made available to the suckling young, or by commercial dairy animals to the milking machine. Milk ejection is brought about by the operation of a reflex process caused by stimulation of the teat. This triggers the nerve receptors in the skin and these nerve impulses ascend the spinal cord to reach the hypothalamus, where they cause release of the hormone oxytocin from the

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posterior lobe of the pituitary gland into the blood circulation (Schmidt, 1971; Hafez, 1974). Oxytocin is carried in the blood to the mammary gland where it causes the myoepithelial cells surrounding the alveoli to contract and expel the milk from the alveoli, forcing it along the duct system toward the gland and teat cisterns and causing the internal pressure in the cisterns to rise (Hafez, 1974; Jacobson, 1975; Bremel 1985; Lollivier *et al*., 2006).

Thus milk ejection occurs as a result of nervous stimulation at the level of the teat and floor of the mammary gland by either the suckling young or the milking machine, followed by transmission of the nerve impulse through the mammary gland and spinal cord to the supraoptic and paraventricular nuclei within the hypothalamus. Stimulation of these hypothalamic nuclei provokes release of oxytocin from its storage site in mangocellular neurons, which extend into the neurohypophysis, and through the posterior pituitary into the systemic circulation (Wakerley *et al*., 1994). In ruminants, 30s after cup attachment and during the first 1–2 min following application of the teat cups, plasma oxytocin concentrations rise. Then they slowly decline, reaching basal oxytocin concentration between 10 and 15 min, suggesting an immediate and short effect on the mammary gland.

In the cow during the interval between milkings, a considerable volume of milk, up to half that secreted, passes into the larger ducts and cisterns where it is readily available to the milker. The remainder of the milk, being the portion stored in the fine ducts and alveoli, cannot be obtained until it is ejected or expelled from these regions into the larger ducts and cisterns (Hafez, 1974). This process of transfer of milk is known as "milk ejection" or the "milk let-down."

In the dairy cow, goat and sheep, the usual stimulus for triggering the milk ejection reflex is the sanitary preparation of the teats and udder before the application of the teat cups. Like other reflexes the milk ejection reflex can become conditioned so that the reflex occurs in response to visual or sound stimuli that the milking animals have come to associate with the act of milking, for instance the appearance of the milker or sight or sound of the milking apparatus. For efficient milking, the cows or goats should be milked as soon as possible after milk ejection has occurred, thereby maintaining a regular routine in preparation for milking, which is important for effective milking. Studies have shown that further releases of oxytocin may occur during milking after initial ejection of milk.

The milk ejection reflex has a significant effect on the quality of milk obtained during milking: alveolar milk is rich in total solids compared with cisternal milk. During machine milking, the first milk removed, corresponding to the cisternal milk, is less rich in milk fat than milk removed at the end of milking, corresponding to the alveolar milk (2.5–5 times more fat compared with cisternal milk). Effectively, milk fat globules with diameters exceeding the internal diameter of the small ducts are transferred from the alveoli to the cistern only as a result of oxytocin-mediated milk ejection (Guinard-Flament *et al*., 2001). Conversely, removal of milk protein and lactose is rather consistent throughout machine milking.

Frequent udder evacuation and milk removal not only reduces the accumulation of feedback inhibitors of lactation but also avoids any deleterious effects of increased intra-alveolar pressure on milk synthesis. Effectively, it has been shown in dairy goats that after 21 hours of milk accumulation, the resulting increased intramammary pressure provokes disruption of the alveolar secretory epithelium via tight junction rupture. Tight junctions begin to become leaky after 18–20 hours of udder filling (Stelwagen & Knight, 1997), which coincides directly with the beginning of decrease of milk secretion rate in dairy goats (Stelwagen *et al*., 1994) and cows (Davis *et al*., 1998). This rupture results in significant modification of the ionic composition of milk, with an increase in milk sodium and chloride and a decrease in milk lactose and potassium (Stelwagen *et al*., 1994). Physiological increases of intramammary pressure cause compression of mammary secretory epithelia, resulting in accompanying mechanical changes within the alveoli including perturbation of the cytoskeleton (Nickerson *et al*., 1980) and mechano-transduction effects on gene expression (Stelwagen, 2001), which all interfere with milk secretion and could lead to apoptosis. Additionally, high intramammary pressure between milking reduces mammary blood flow by 10% after 24 hours of udder filling in dairy cows (Guinard-Flament & Rulquin, 2001) and by 50% after 36 hours in dairy goats (Stelwagen *et al*., 1994), which at the same time reduces the supply of nutrition and hormones to the udder and then reduces the rate of milk secretion (Peaker, 1980). Alternatively, milk removal from the gland during machine milking improves mammary blood flow in dairy cows (Houvenaghel *et al*., 1973) and in dairy goats (Reynolds *et al*., 1968).

2.5 MAINTENANCE OF LACTATION (GALACTOPOIESIS)

Once lactation has been established, it can be maintained for long periods, especially in ruminants, as long as the conditions within the animal do not reduce lactation (Hafez, 1974). The need for adequate food, maintenance of health and regular milking are self-evident factors concerned with adequate husbandry, but the hormonal factors necessary for the maintenance of lactation require much more elaboration.

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The maintenance of lactation or milk secretion is dependent upon the milking or suckling stimulus, which is involved in the release of prolactin, corticotrophin (ACTH) and oxytocin from the pituitary gland. Additionally, the suckling or milking process is needed to remove the milk from the gland, because removal is required for further milk synthesis to take place. It appears that the goat is an exception to the group of animals that requires suckling or milking stimulus for the maintenance of milk secretion (Schmidt, 1971). Failure to remove the milk causes pressure to build up within the gland, resulting in cessation of secretion and the beginning of involution. The suckling stimulus can maintain the secretory activity of glands, and also can prolong lactation for long periods.

After parturition, milk yield rises rapidly and then declines slowly until the young are weaned. The hormonal mechanisms involved in the maintenance of lactation (galactopoiesis) are very similar to those involved in the initiation of milk production. Hypophysectomy at any period during lactation terminates the milk secretion and galactopoiesis process. The continued production of prolactin is likely to be essential throughout the period of lactation, and certainly ACTH, somatotrophin (STH), and thyrotrophin (TSH) are also of importance in galactopoiesis. The hormones involved in lactogenesis as well as maintenance of lactation in rats are displayed in Fig. 2.4, which resembles the situation in ruminant dairy species. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) function synergistically to promote the secretion of estrogen by the ovary (Schmidt, 1971; Hafez, 1974; Knight, 1993). Figure 2.4 also indicates that ACTH stimulates the adrenal cortex to secrete corticoids, and growth of the duct system is initiated by the action of estrogen, STH and adrenal corticoids. Prolactin stimulates the corpora lutea to secrete progesterone. Full lobulo-alveolar (prolactational) development requires a combination of prolactin, STH, estrogen, progesterone and corticoids. Milk secretion by the developed gland ensues when the influence of estrogen and progesterone is diminished and prolactin and adrenal corticoids attain supremacy. Lactogenesis is facilitated probably by STH and TSH, although neither is absolutely necessary in the rat (Lyons *et al*., 1958; Akers *et al*., 2005).

When milk production begins, pressure develops within the glands and, if not relieved by suckling or milking, rises to the point where milk secretion is retarded. The mammary glands of mice and rats involute quickly after removal of the litters, but the administration of prolactin to such animals tends to maintain the alveolar epithelium and retard involution (Turner & Bagnara, 1971). When the young are removed from rats on the fourth day of lactation, injections of oxytocin markedly delay mammary involution. Ablation of the neural lobe of the hypophysis of lactating rats abolishes the milk ejection reflex, and the young die from starvation unless oxytocin is injected into the mother (Turner & Bagnara, 1971).

As shown in Fig. 2.4, stimulation of the mammary gland during nursing and machine milking is of great benefit to the gland due to the release of several lactogenic hormones from the pituitary. Prolactin favors the synthesis and secretion of the milk components, and also influences fat metabolism within mammary adipose tissue (Kann *et al*., 1977). ACTH released during milking participates in conjunction with the glucocorticoids in maintaining lactation via its general effects on metabolism, by markedly amplifying the gland's response to prolactin (Hobbs *et al*., 1982). It was originally suggested that growth hormone (GH) is released during milking in goats (Hart & Flux, 1973), and the simultaneous effect of administration of GH and increasing the milking frequency was additive, indicating that these two galactopoietic stimuli operate by independent mechanisms. Thus, increased milking frequency could assure a better hormonal maintenance of lactation simply as a result of increased stimulation of hormonal release, and thus more metabolic and synthetic activity within the mammary gland.

These hormones could improve the number of secretory cells in addition to their metabolic and endocrine effects, thus increasing the volume of milk secreted (Hobbs *et al*., 1982; Knight, 1993). In ruminants, the number of secretory cells slowly decreases throughout the course of an established lactation via apoptotic mechanisms (Quarrie *et al*., 1995), without modification of the secretory activities of the remaining cells (Knight, 1993). The diminution in the number of secretory cells is not predetermined and instead can be modified by milking frequency (Li *et al*., 1999). Increasing the number of daily milkings results in cellular hypertrophy, which is followed by proliferation of new secretory cells and then by an increase in secretory cell numbers. This proliferative effect permits an increase in lactation persistency with little effect on milk composition (Hillerton *et al*., 1990).

Machine milking or nursing, as indicated previously, is the principal stimulus responsible for the release of oxytocin from the posterior pituitary, and for the subsequent oxytocin-mediated myoepithelial contraction for milk removal. For many years, this reflex has been considered the only mechanism within the mammary gland able to elicit milk ejection. Pituitary oxytocin is thus generally considered as the only hormone able to increase milk production via stimulation of the milk ejection reflex (Knight, 1994). In addition to neurohypophyseal release of oxytocin during machine milking, oxytocin is synthesized, stored, and released by the corpus luteum (Swann *et al*., 1984), which appears cyclically throughout estrous seasons yet

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independent of lactation and udder or breast stimulation. This luteal oxytocin may also participate in facilitating milk removal by its influence on milk transfer from the alveoli to the cistern between milking (McKusick *et al*., 2002a).

The increase in milk production associated with injection or natural release of oxytocin can only be explained by a mechanical action of oxytocin as a result of myoepithelial contraction and thus expulsion of the synthesized milk from the alveoli (Da Costa *et al*., 1995), thereby limiting the negative effects of feedback inhibitors of lactation or increased intra-alveolar pressure on milk secretion. Nevertheless, the galactopoietic action of oxytocin could also be explained by direct stimulation of mammary blood flow (Fleet *et al*., 1993), probably as a result of the vasopressin-like effect of oxytocin, which would increase the supply of nutrients and lactogenic hormones to the gland.

Another possibility for the increase in milk production by natural release of oxytocin is that oxytocin may exert a direct effect on the epithelial cell. For instance, Nostrand *et al*. (1991) demonstrated that long-term daily injections of oxytocin in dairy cows resulted in an increase in milk production, particularly during the descending phase of lactation. These results might be explained by a galactopoietic effect of oxytocin. Moreover, oxytocin may act on mammary cells by inducing cell differentiation and proliferation as was shown in nonlactating mouse mammary glands (Sapino *et al*., 1993). Finally, a direct galactopoietic effect of oxytocin on milk synthesis in the mammary gland is probable, which might explain the increases in milk production (Ballou *et al*., 1993). Ollivier-Bousquet (1976) demonstrated that *in vitro*, addition of oxytocin resulted in acceleration of intracellular transit of casein as well as augmentation of its secretion in lactating rabbit mammary fragments. Increases in secretion rate of casein were not simply due to a mechanical effect of myoepithelial contraction, because intracellular transport of nascent protein between the endoplasmic reticulum, Golgi apparatus, and secretory vesicles was also affected.

If oxytocin is administered over medium or long periods, there is no modification of milk fat, protein, lactose, somatic cell count, or plasmin activity (Nostrand *et al*., 1991). However, oxytocin administration has been shown to significantly modify milk quality, and the effect is proportional to dose: increasing doses (0.1–3 IU) result in increases in milk fat, but concurrent decreases in milk protein (Sagi *et al*., 1980). Additionally, massive doses of oxytocin (40 IU every 20 min for 80 min following milking) reduce milk fat content without affecting milk fatty acid composition (Dill *et al*., 1974). Finally, oxytocin administration could have a negative effect on milk fat content by retaining milk fat within the alveolar compartment. Indeed, two injections of oxytocin 10 IU, without milk removal, significantly increased fat globule diameter by 0.22 μm (4.25 to 4.47 μm) and the resulting increase in intramammary pressure might induce fat globule coalescence (Guinard-Flament *et al*., 2001).

During machine milking, the cisternal milk is immediately available and can be obtained independently of milk ejection; cisternal milk represents approximately 20% of the total milk volume in dairy cows after a normal 12-hour milking interval (Pfeilsticker *et al*., 1996), and 50–80% in dairy ewes (McKusick *et al*., 2002b) and goats (Marnet & McKusick, 2001). In contrast, the alveolar milk, which remains fixed in the mammary gland by capillary forces and by the presence of sphincters in the small intralobular ducts (Zaks, 1962), is only obtained when active expulsion of milk occurs during the oxytocin-mediated milk ejection reflex.

2.6 SECRETION OF MILK AND ITS CONSTITUENTS

2.6.1 Types of milk secretion

There are three types of milk secretion within the mammary gland. Merocrine type secretion involves movement of the secretion products through the epithelial cell membrane without injury to the membrane itself. This type of milk secretion occurs in cows, and the secretion of milk particles does not damage the cell membrane. The myoepithelial cell has no secretory function and is involved primarily in contraction of the alveolus during milk ejection.

Holocrine secretion is the type of milk secretion where the entire epithelial cell disintegrates to become part of the secretion. It does not apply to cow milk secretion, and this is not an important form of milk secretion, because the epithelial cells do not divide rapidly enough to produce a large volume of milk. The presence of cytoplasmic fragments and nuclei in the milk can best be explained by a daily disintegration of some epithelial cells during the course of lactation (Schmidt, 1971).

The apocrine type of secretion involves the migration of the secretory products to the apex of the epithelial cell, where rupture of the cell membrane occurs to release the secretory products. With this type of secretion, part of the cytoplasm is exposed and a small part of it is released with the secretory droplets. Goat milk is produced mainly by apocrine type secretion and probably also by some holocrine secretion. This is the reason why goat milk has naturally high somatic cell counts compared with cow milk, even when the goats are free of mastitis or have no apparent infection in the mammary glands. This phenomenon explains why normal goat milk contains a large amount of disintegrated epithelial cell contents such as cytoplasmic fragments and nuclei (Dulin *et al*., 1982; Park, 1991).

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2.6.2 Milk secretion process

The constituents of milk are produced by the epithelial cells of alveoli in two ways: synthesis and diffusion. One group of compounds, which includes milk fat, most of the protein components and lactose, is synthesized in the epithelial cells from blood precursors and then released into the lumen of the alveolus. The remaining milk constituents, such as water, minerals and vitamins, pass from the blood and move across the epithelial cells or between them into the alveolar lumina without alteration by the cells via the diffusion process (Schmidt, 1971).

Approximately 500 volumes of blood flow through the udder of the cow and goat for each volume of milk produced. The ratio of blood flow to milk yield is higher in lower-producing goats and in animals in late lactation. The blood flow to milk yield ratio increases with advancing lactation and with a drop in yield due to illness (Linzell, 1960). The blood precursors of ruminant milk constituents are shown in Table 2.1.

Lactose is synthesized primarily from glucose, while the major milk proteins, the caseins and the whey proteins β-lactoglobulin and α-lactalbumin, are synthesized from amino acids. In the ruminant, acetate and β-hydroxybutyrate are precursors of fatty acids up to C16 (palmitic acid) in length. A small portion of the C12 and C14 fatty acids arise from blood C16 fatty acids (Barry, 1964). Most of the stearic acid (C18) comes from triglycerides in the blood and is not built up from C2 or C4 units. Most of the unsaturated fatty acids in the milk are derived from dehydrogenation of the saturated fatty acids, such as stearic acid. The mammary gland cannot convert an unsaturated fatty acid to a saturated fatty acid, for example oleic acid cannot be converted to stearic acid. Glycerol is mainly synthesized from glucose in the mammary gland, and a small part comes from the triglycerides absorbed from the blood. On the other hand, glucose is the primary precursor of the fatty acids in the milk of nonruminant species (Barry, 1964).

2.6.3 Comparative composition of blood and milk nutrients

The composition of blood plasma and that of cows' milk is shown in Table 2.2. Milk contains much higher concentrations of carbohydrates and fats than blood. The same trend is also true for calcium and phosphorus. Milk has a lower protein content, especially albumin and globulin, than blood, while milk has a much higher concentration of casein which is almost negligible in blood. Blood plasma also contains considerably higher sodium and chlorine than milk. Since the variation in mineral content in milk is very small, the mammary gland epithelial cells must regulate the mineral composition in some way.

In goats, it has been shown that 100% of the calcium and 80% of the nitrogen and calories removed from the blood by the udder appear in its milk. In addition, 80% of the neutral fat and volatile fatty acids taken up by the mammary gland also appear in milk fat. On the other hand, only 50% of the glucose appears as lactose, while the remaining glucose is used for energy. As a source of energy, acetate is also used for the ruminant mammary gland (Linzell, 1960).

Table 2.1. Blood precursors of the milk constituents in the ruminant.

Milk constituent	Blood precursor
Water	Water
Lactose	Glucose
Protein	
Caseins	Amino acids
β-Lactoglobulin	Amino acids
α-Lactalbumin	Amino acids
Milk serum albumin	Amino acids
Immunoglobulins	Immunoglobulins
Fat	
Fatty acids	Acetate, β-hydroxybutyrate, blood lipids
Glycerol	Glucose, glycerol from triglycerides
Minerals	Minerals
Vitamins	Vitamins

Source: based on data from Schmidt (1971).

Table 2.2. Comparative composition of blood plasma and milk of the cow.

Blood plasma	Milk
Water, 91.0%	Water, 87.0%
Glucose, 0.05%	Lactose, 4.90%
	Caseins, 2.90%
Serum albumin, 3.20%	α-Lactalbumin, 0.52%
Serum globulin, 4.40%	β-Lactoglobulin, 0.20%
Neutral fat, 0.06%	Neutral fat, 3.70%
Phospholipids, 0.24%	Phospholipids, 0.10%
Calcium, 0.009%	Calcium, 0.12%
Phosphorus, 0.011%	Phosphorus, 0.10%
Sodium, 0.34%	Sodium, 0.05%
Potassium, 0.03%	Potassium, 0.15%
Chlorine, 0.35%	Chlorine, 0.11%
Citric acid, trace	Citric acid, 0.20%

Source: based on data from Schmidt (1971).

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First-calf heifers with noninfected mammary glands have a constant amount of sodium, potassium, and lactose in their milk. Within a breed, there is a close inverse relationship between lactose content and the molar sums of potassium and sodium concentrations in the milk. This phenomenon has led to the hypothesis that the water in milk secreted by the alveolar cells arises in two ways: part arises in the secretory cell as water of the potassium-rich intracellular fluid; and part arises as a result of the synthesis of lactose, proteins and fat, since water moves into the cell to maintain osmotic equilibrium. These two portions are constant within an individual but vary between animals (Schmidt, 1971). The osmotic pressure of milk constituents is primarily dependent on the concentration of lactose and sodium and potassium and their associated anions. More than one-third of the osmotic pressure of normal milk is attributable to lactose content alone. In addition to the primary secretion of water in milk, a blood plasma transudate rich in sodium and chlorine contributes to the fluid of milk. This type of secretion appears to be important in milk secretion during advancing lactation, advancing age and bacterial infection of the udder. Milk secreted during these periods contains increased levels of sodium and chlorine and decreased levels of lactose and potassium (Rook & Wood, 1958; Wheelock & Rook, 1966; Rook & Wheelock, 1967).

2.7 INVOLUTION OF THE MAMMARY GLAND

The mammary gland, although physiologically considered a powerful machine, is constantly faced with the juxtaposition of producing sufficient amounts of milk (originally destined for nutrition of the young), yet adequately ridding itself of this milk in order to avoid the negative effects of milk stagnation on milk synthesis and on eventual involution of the gland. In order to avoid over-production, which would be a waste of valuable metabolites that could be used for other life processes (reproduction, for example), the mammary gland is equipped with an autoregulatory mechanism for the control of milk synthesis. Frequent and complete milk removal reduces the negative effect of this self-regulatory mechanism on milk secretion. This mechanism has been associated with the cosecretion of a milk factor with autocrine activity. The first of these factors to be described was a glycoprotein that has the ability to exert negative feedback locally on milk protein and lactose synthesis. This glycoprotein (known as the feedback inhibitor of lactation, FIL) was first described in the goat (Wilde *et al*., 1995), because of its ability to reduce the rate of milk secretion *in vitro* (Wilde *et al*., 1987, 1989) and *in vivo* (Wilde *et al*., 1988) when in contact with the alveolar epithelium (Peaker & Blatchford, 1988). FIL reversibly blocks the constitutive pathway of milk protein biosynthesis (specifically transport between the endoplasmic reticulum and the Golgi apparatus, as well as intra-Golgi transport) in mammary epithelial cells, resulting in inhibition of casein and lactose synthesis, as well as stimulation of the intracellular degradation of newly synthesized casein (Wilde *et al*., 1989). In addition, FIL regulates the size of the secretory cell population, in part by triggering apoptosis of epithelial cells in the mammary gland (Quarrie *et al*., 1995) and/or by reducing the sensitivity of mammary epithelial cells to galactopoietic hormones such as prolactin via a reduction in the number of prolactin receptors (Bennett *et al*., 1990). A reduction in prolactin receptors is thought to reduce the normal anti-apoptotic properties of prolactin (Travers *et al*., 1996; Flint & Knight, 1997; Tonner *et al*., 2000). FIL also probably reduces the synthesis and secretion of mammary cells by blocking the potassium channel of the apical membrane (Silanikove *et al*., 2000). Shamay *et al*. (2002) hypothesized that FIL can be identified with amino acid sequence 1–28 derived from the breakdown of β-casein by plasmin, and this was confirmed by Pulina *et al*. (2005) in goats. Finally, when machine milking is incomplete, or when the daily number of milkings is reduced, accumulation of FIL within the alveoli increases, which leads to reduced milk synthesis as described in this paragraph.

The peak yield in milk secretion usually occurs within a few days or weeks after parturition in most species, followed by a decrease in milk secretion. This decrease is mainly due to involution of the mammary gland tissue. Involution results in a decrease in the number of milk secretory cells in the mammary gland tissue. Part of the reduction in milk production following peak lactation may also be attributed to a reduction in secretion rate for each remaining cell.

Complete involution of secretory tissue usually occurs after the cessation of milking or the suckling of young (Schmidt, 1971; Hafez, 1974). This kind of induced involution occurs as a much more drastic form of involution than the process that takes place during lactation. When the milking or suckling of young stops, there is a very rapid disappearance of the secretory cells and degeneration of the alveolar and lobular structures. The efficiency of milk production by the dairy industry could be greatly and positively affected if the involution process in milking animals could be reduced or eliminated by hormonal, nutritional, environmental or other factors (Schmidt, 1971).

As involution progresses, regression of parenchymal tissue of the udder may be a rapid or slow process depending on the specific circumstances. The regression of tissue will occur rapidly if milk removal is suddenly stopped in fully lactating animals, although it is usually a gradual process associated with the natural decline in milk yield. However, the process will become somewhat accelerated when

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weaning finally occurs or milk production ceases (Hafez, 1974).

During involution, the histological changes in the cow, goat and guinea pig are characterized by a decrease in the size of the alveoli, a decrease in the number of alveoli per lobule, a decrease in the total number of alveoli and lobular volume, and a decrease in the number of cells per alveolus (Schmidt, 1971; Hafez, 1974). Complete lobules also disintegrate in parts of the mammary gland during advancing involution. At the end of involution, the gland takes on the appearance of that of the virgin animal, but the essential lobular structure of the gland is still recognizable (Schmidt, 1971).

The histological and cytological changes in the acute or rapid type of mammary regression have been extensively studied in laboratory animals and to a lesser extent in ruminants (Hafez, 1974). These investigations have shown that the alveoli soon become distended, their walls stretched and the alveolar cells flattened, the capillaries compressed, and the blood supply greatly reduced (Hafez, 1974). Within 3–4 days the alveolar cells break up, lysosomal hydrolytic enzymes are released, and there is digestion of the cellular components and resorption of the secretory products, which diffuse into the interstitial spaces and are carried away in the lymph. By day 5 the alveoli collapse and disappear and the gland is infiltrated by phagocytic cells. Macrophages play a major role in the removal of the fat from the regressing gland in ruminants. The lobular structure eventually disappears, the stroma predominates, and the parenchyma is reduced to a duct system (Hafez, 1974). The tissue and cellular changes occur with the "drying off" of the udder at the end of lactation, but few studies have reported on this aspect.

When regular milking is continued, both the amount of parenchyma and its secretory activity decline in the course of normal lactation. The decline in yield is more noticeable if a new pregnancy supervenes because the resulting hormonal patterns of raised estrogen and progesterone levels will tend to depress secretory activity, while maintaining or even stimulating parenchymal growth. There is little evidence that any marked regression occurs in the lobulo-alveolar tissue of the lactating cow that is pregnant and which is "dried off" 2 months before parturition (Hafez, 1974).

During involution, the loss of epithelial cells occurs with a decrease in the rate of activity per cell. Biochemical alterations occur within hours after the cessation of milking or suckling, comprising a decrease in the respiratory quotient, a decrease in oxygen consumption, and an accumulation of lactic acid in the mammary gland tissue. Within 12–24 hours after the young have been removed, oxidative phosphorylation of the mitochondria of the mammary gland is uncoupled (Schmidt, 1971). The involution process is prevented by prolactin injection, and the injection maintains the biochemical integrity of the mammary gland to a greater extent than oxytocin injections. However, prolactin will not maintain full biochemical integrity of the tissue unless the secretory products are removed. It is known that several drugs and hormones, such as reserpine, acetylcholine, serotonin and epinephrine, also prevent involution of the mammary gland after the cessation of suckling. These drug and hormone compounds exert their effects by causing prolactin secretion from the pituitary gland (Schmidt, 1971).

2.8 CHALLENGES AND OPPORTUNITIES IN MAMMARY SECRETION TODAY AND TOMORROW

One of the great challenges for the dairy industry around the world today and tomorrow is how to feed the increasing human population sufficiently (Haenlein & Abdellatif, 2004). The dairy cow, specifically the US Holstein cow, can be taken as a model for what is possible in achieving high-yielding dairy animals. During the last 50 years, US Holsteins have more than doubled their annual milk yields to above 10 000 kg (>20 000 lb) by producing more than 50 kg (>100 lb) per day during peak lactation. Similar improvements in mammary secretion have also been achieved by the other US dairy breeds (Jersey, Brown Swiss, Guernsey, Ayrshire) because of concerted efforts by the National Cooperative Dairy Herd Improvement Program to record individual performance for genetic selection of superior sires and dams in milk yield and in classification of body type, especially for improved udder capacity, attachment and teat shape (Majeskie, 1986).

Similar efforts in record-keeping and classification have also succeeded in the selection of greatly improved US dairy goats, particularly their productivity and udder and teat shape (Lawrence & Murrill, 1986; Wiggans, 1986; Haenlein, 2007). Average annual official milk production records of US Saanen in 2006 were 914 kg (846 kg 4% fat-corrected milk, FCM), of US Alpine 869 kg (813 kg FCM), and of US Nubian 710 kg (764 kg FCM), but individual maximums were 3620 kg for a US Toggenburg, 2916 kg for a US Alpine, and 2700 kg for a US Nubian, all in 10-month lactations. This compares with the generally low productivity of dairy goats in other countries, presently averaging around 100–200 kg in less than 10-month lactations, except for some higher levels in Switzerland, France, and Spain (Haenlein & Abdellatif, 2004). However, it is evident that there is great potential for improved mammary secretion levels in dairy goats in other countries, where greater food supplies from local production are urgently needed. The situation for dairy sheep is similar, with German East

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Friesian and Israeli Awassi averaging in excess of 600 and 500 kg, respectively, while in other countries the average yields per ewe are between 50 and 100 kg in lactations of only 5–7 months.

Attention to better udder and teat conformation is a particularly urgent challenge in most dairy sheep breeds, where the teat is located on the side of the udder and the gland cistern is lower than the position of the teats, making complete emptying of the mammary secretions difficult without using after-stripping. Since udder shapes and teat placement are hereditary traits, progress through selection is possible. Likewise the short lactation lengths in dairy sheep should greatly improve through genetic selection and more appropriate nutrition. Similarly, genetic selection should also increase the very small gland cistern and teat size in mares, donkeys and camels used for milk production.

Another challenge is the continued need for the presence of the foal and calf when mares or Zebu cows are to be milked. Again, genetic selection should succeed in providing the oxytocin milk-let down effect without requiring the presence of the foal or calf.

Controlling health concerns, especially of tuberculosis and brucellosis in dairy water buffaloes, is an urgent challenge for quality and healthy milk, which can be accomplished as is evident in the dairy cattle populations of Western countries.

Finally, the dairy goat industry now has the opportunity to test goat milk for the presence and amount of α_{s1}-casein and α_{s2}-casein in order to accommodate market needs, since they differ significantly in cheese-making characteristics and yields, but also apparently in sensitivity against cow milk allergy and digestibility in human nutrition (Moioli *et al*., 2007).

More attention to these challenges and opportunities should provide better supplies of dairy products to the growing human population around the world, where local production is widely insufficient. In Egypt, for example, total milk production increased by 46% during the 1990s but milk consumption increased by 73%, leaving a domestic shortfall of 2 081 000 t in 1998 (Haenlein & Abdellatif, 2004).

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3
Milking Procedures and Facilities

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3.1 INTRODUCTION

Mechanical milking is today an inevitable part of regular high-yielding milk production because it allows more animals to be milked per milker per hour in better working conditions than achievable by manual milking. Even in less widespread dairy species (sheep, goats, yaks, buffaloes, camels, horses, zebu, reindeer) bred in less economically developed countries where only traditional small-scale milking exists (Mediterranean region, Balkan Peninsula, Saudi Arabia, Near East, India, Asia Minor, China, Mongolia, Tibet, Siberia, Lapland), machine milking has begun to develop. Machine milking permits the harvesting of better-quality hygienic milk, better adapted for processing and preservation and for commercialization.

Nevertheless, machine milking does not just involve milk harvesting but is a much more complex operation that requires respect for and understanding of animals and their active participation. Good stimulation of milk let-down depends on a well-designed system of machine milking, otherwise the milk collected will be limited in volume and of variable quality, and the milked animals will retain part of the milk, the so-called residual milk, in the upper part of the mammary gland (see Chapter 2 for animal lactation physiology). Finally, milking can be performed at various intervals depending on tradition (e.g., once-daily milking of goats in the Canary Islands), cost of labor (thrice-daily milkings in the USA and China), and the expected milk productivity or milking technology (free access to milking in robotic milking systems).

After a brief description of machine-milked animals throughout the world, this chapter describes the principles of machine milking, the effects of milking materials and setting, and the effects of milking practices and systems on milk quantity and quality.

3.2 MACHINE MILKED ANIMALS THROUGHOUT THE WORLD

While the milking machine was developed for cows and the main principles laid down at the beginning of the twentieth century, it was only later adapted to other species. In France, where milking of small ruminants is an ancient tradition, machine milking of Lacaune dairy sheep (for Roquefort cheese production) only developed in the 1960s with the help of a French professor, Jacques Labussière. He first described the better milking conditions for and milking ability of these animals by measuring milk flow during milking (Labussière *et al*., 1964). With the active cooperation of members of the milk-producing community, he developed specific milking parlors ("Casse" type with a mobile fence specifically developed to milk dairy ewes) and organized training of shepherd milkers. Machine milking of dairy goats (Fig. 3.1 and see Plate 3.1) developed later, but for a long time milking was done with the equipment and settings used for cows. The better milking ability of cows delayed the development of specific materials and parlors, although specific improvements did occur when herd sizes increased and caused concerns about the workload and milk quality. Gradually, over the last 30 years, humans have tried to machine milk other species that were traditionally hand milked for liquid milk or the manufacture of yoghurt, cheese, butter, soap and cosmetics. Specific parlors, clusters, and liners have now been developed

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Figure 3.1. Simple platform, side-by-side, milking parlor for goats at the AGROCAMPUS OUEST/INRA laboratory. For a color version of this figure, see Plate 3.1.



Figure 3.2. Buffalo milking with autonomous bucket machine milking. For a color version of this figure, see Plate 3.2.

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for milking water buffalo (Fig. 3.2 and see Plate 3.2) in India and Pakistan and other Asian countries mainly, or Zebu and numerous crossbreeds (Australian Zebu, Gyr cows in Brazil, Mithun in India). Other species are more difficult to milk because the young calf or foal is required to be present during milking, or because of the small cistern storage capacity of their udder requiring three to five milking episodes per day; however, these species are now also machine milked for scarce and expensive products sold to niche markets. This is the case for horses, whose milk is used to produce a fermented milk traditionally made in Mongolia and southeastern Russia but now appreciated also by the Turkish population of Germany (Koumiss), or for cosmetic products (creams, soaps). Today, machine milking of camels (Fig. 3.3 and see Plate 3.3) is booming in African and Arabian countries. There have also been a few reports about recent developments regarding machine milking of yaks in China, of reindeer and even of moose by the Siberian populations of Russia and Laplanders in northern Scandinavia, but little is known about the milking ability of these species and about better adapted equipment and settings for efficient milking. Some applied research work is thus still needed to help these technical developments and in other species now exclusively hand milked, like donkeys. It is aparent that all terrestrial mammals can be machine milked when their physiological, anatomical, and behavioral specificities are taken into account. There have even been reports of the milking of sows and deer for experimental purposes.

3.3 MILKING PRINCIPLES

First of all, it is very important to remember that suckling of ruminants is based on the conjunction of two mechanisms:

1. A suckling calf wraps its tongue around the teat. A high vacuum is created at the tip of the teat when the jaws widen and the tongue presses the teat against the roof of the mouth and retracts toward the throat at a frequency of 2–2.4 Hz. High vacuum pressure in the mouth and regular and high-frequency tongue pressure on the roof of the mouth increases the pressure in the teat cistern, inducing the teat sphincter to open (the differential pressure between the cistern of the teat and the mouth vacuum can reach 110 kPa, as described by Rasmussen & Maintz, 1998) and the teat cistern milk is ejected in regular spurts. When the calf swallows the milk, the vacuum in the mouth returns to air pressure.
2. Widely used throughout the world, hand milking uses only pressure. The hand grasps the whole length of the teat. The thumb and forefinger pinch off the upper end of the teat as the other fingers squeeze inward and downward at an approximate frequency of 1 Hz (McDonald & Witzel, 1965). The increased pressure inside the teat forces the milk out through the sphincter. When the teat is very short, as with the udders of ewes, mares and jennies, full hand milking is replaced by stripping with the thumb and forefinger.



Figure 3.3. Camel milking stall and equipment in Saudi Arabia. Courtesy of Dr B. Faye. For a color version of this figure, see Plate 3.3.

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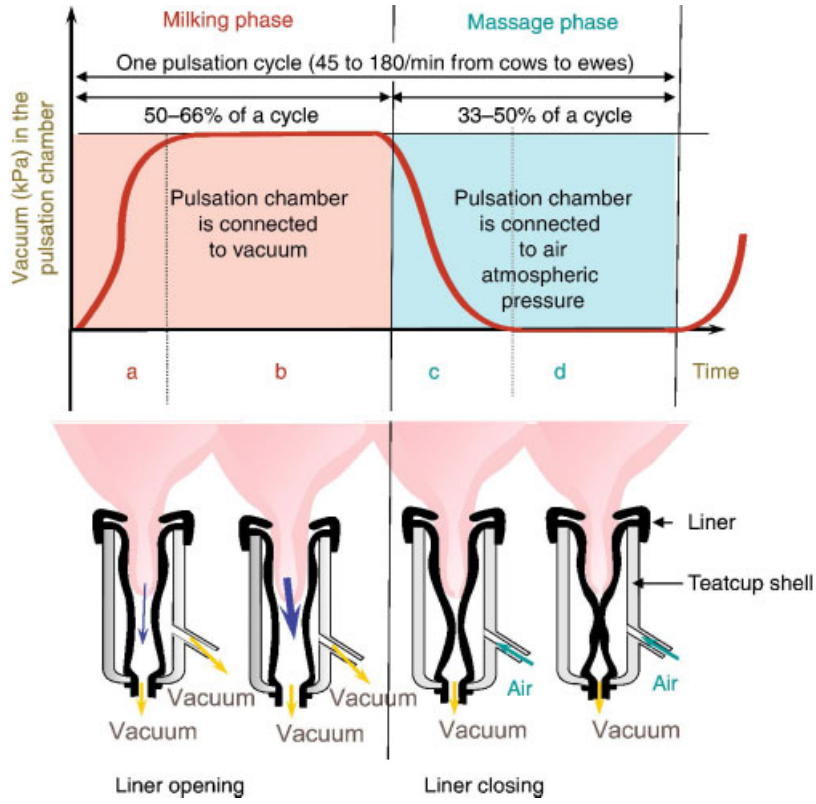


Figure 3.4. Principle of machine milking and action of the liner in the teat cup during machine milking. Reproduced from Wattiaux (2004–2011), with permission of Babcock Research Institute (http://babcock.wisc.edu/node/206).

The milking machine uses only vacuum to extract milk from the udder. Nevertheless, if a high-level vacuum is maintained under the teat continuously, blood and body fluids can accumulate in the teat tissue, resulting in teat congestion and edema and reducing milk flow. This is the reason for the development of a pulsation system permitted by the development of the double-chambered teat cup (Fig. 3.4). When the pressure inside the chamber and the pressure inside the vacuum line are the same, the liner is open (sucking of milking phase). The differential pressure between the inside of the teat and liner forces the teat sphincter to open and milk is ejected out of the teat cistern into the line. The pulsator is a device that allows air pressure and vacuum to enter the chamber alternately. However, when the pulsator switches to air, the pressure inside the liner becomes lower than inside the pulsation chamber and the liner collapses beneath the teat, closing the teat canal and stopping milk flow (massage phase). This massaging action of the liner during a pulsation cycle prevents fluid congestion and edema of the teat.

On this basis, three main parameters control the settings of the machine milking and these impact milk ejection and quality: vacuum level (controlled by a pumping device and vacuum regulator); pulsation rate (frequency of a complete cycle of milking and massage phases, e.g., 60 pulses/min for cows, 90 or 120 pulses/min for goats, 180 pulses/min for ewes); and pulsator ratio (time reserved for sucking phase related to massage phase). The pulsation parameters are controlled by a pneumatic or electronic pulsator.

3.4 MILKING MACHINE COMPONENTS AND EFFECTS ON MILK HARVESTING AND QUALITY

All milking machines, from single-unit bucket machines to multi-unit herringbone, side-by-side or tandem parlors and from rotary platform systems to automatic robotic milking systems (AMS), have the same basic elements (Figs 3.4 and 3.5): the vacuum system, the pulsation components, the milking cluster (except on robots where the system is simplified without a claw), and a pipe, glass jar and tank arrangement for transporting, measuring, releasing, and storing the milk.

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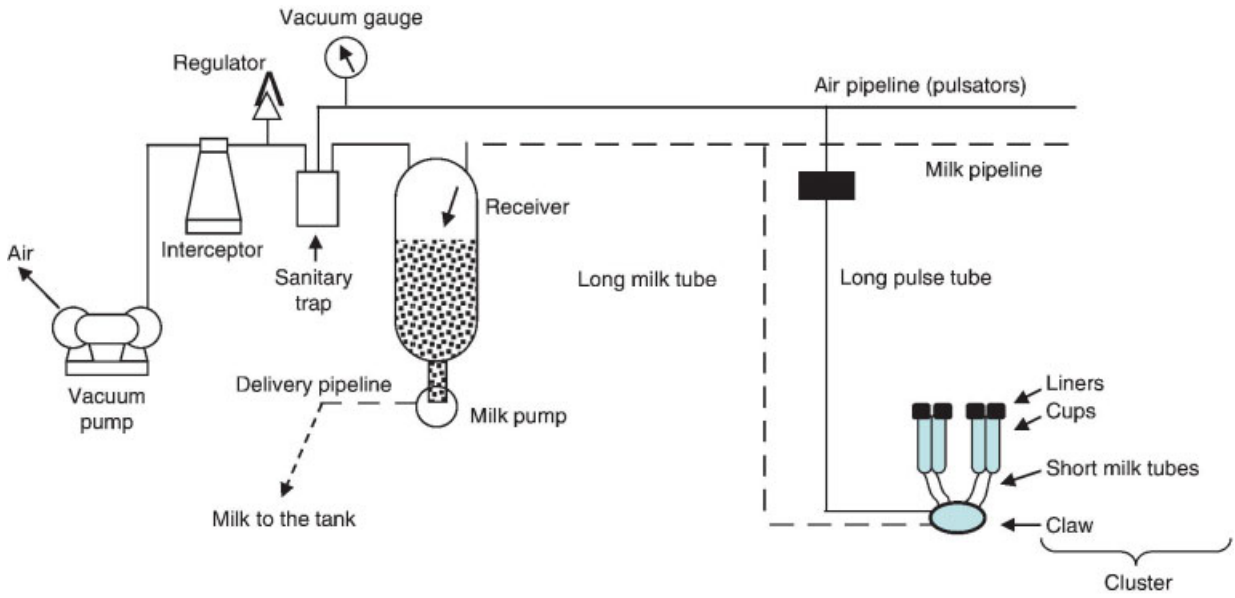


Figure 3.5. Example of machine milking installation with milk pipeline (cowshed and parlor). Reproduced from FAO (1989), with permission.

3.4.1 Vacuum system

Vacuum is regulated and must be as stable as possible under the teat. To achieve this, the pump needs a sufficient vacuum capacity to cover basal vacuum consumption (normal air entry at the claw or teat cup vent level, pulsator and during cluster removal/automatic take-off, and some unavoidable vacuum leakage along the vacuum line) and acyclic vacuum variation due to cluster manipulation (air entry during teat cup attachment) of because of animal and cluster movements (liner slipping, kicking). A sensitive regulator controls air entry to avoid excessive vacuum or compensates for acyclic fluctuations in vacuum. If the vacuum reserve is not sufficient or the regulator not sensitive enough, vacuum fluctuations under the teat can be deleterious, leading to liner slippage (liner squawks) and sometimes to the machine falling off the cow. This sudden large admission of air into the liner can reverse the flow of milk in the claw, leading to the impaction of milk droplets on the end of contralateral teats and sometimes to injection of milk into the teat cistern. Such a malfunction increases the risk of udder inflammation (rise in somatic cell count, SCC) and may give rise to bacterial invasion of the teat cistern and mastitis (Thiel *et al*., 1973). The frequency of impaction increases with improper application or removal of teat cups, difficult teat placement, vigorous machine stripping, and inadequate positioning of the milking unit under the animal. Vacuum fluctuations in the claw and consecutive impacts are reduced with low milk lines compared with high milk lines (Thompson & Pearson, 1979). A reverse pressure gradient occurs when the vacuum in the teat cistern is higher than beneath the teat end for short periods, such as during milking of empty teats (Rasmussen *et al*., 1994). Over-milking (vacuum application when milk flow is reduced or null) will increase the possibility of bacteria entering the teat and causing infections.

The level of vacuum is important, allowing the cluster to remain in place (thus a heavy cluster needs a higher vacuum level than a light one), while an excessively low vacuum leads to an increasing risk of the cluster slipping and falling off during milking. Mean vacuum in the short milk tube should not be lower than 32 kPa as recommended by Rasmussen and Madsen (2000). Vacuum allows extraction and drainage of milk throughout the claw to the milk line. Nevertheless, if the vacuum level inside the cluster is too high or the cluster is too light, the cluster "creeps up" and tends to pinch the area where the teat meets the udder. Milk flow stops and the operator must pull the unit down to completely milk out the cow (machine stripping). If a high vacuum shortens the milking duration, it becomes a risk factor for integrity of the teat end. During a normal pulsation cycle, teat thickness increases during the low-flow period but decreases during the high-flow period (Mein *et al*., 1973). When the vacuum level is too high and/or when the milk flow has ceased, massage of the teat may be

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insufficient and the teat end could become increasingly congested, limiting milk flow rate and negating all the benefits on milking time. If a high vacuum is maintained for a long period, teat canal extraversion and hyperkeratosis occur together with the problem of reduced closing efficiency of the teat sphincter, which usually prevents bacterial invasion of the teat cistern (Hamann, 1990). If milk extraction has been difficult, the milked volume is reduced and stripping volume increases. According to Ebendorff *et al*. (1987) or Hamann and Dodd (1992), leaving an average of 0.55 kg of stripping milk per half udder per day reduced milk yield in the nonstripped half udders by about 6–10% over lactations in mature cows. This milk, retained in the udder if stripping is omitted, can increase risk of bacterial multiplication until the next milking and the risk of white cell recruitment, SCC increase and mastitis as reported in the review of O'Shea (1987). It will be noticed that a high SCC can significantly increase the proteolytic activity (plasmin and cathepsin D mainly) in milk and lead to caseinolysis, with a proportional reduction in cheese yield, loss of casein in whey and slower rate of curd formation (Leroux *et al*., 2003). A high SCC can also induce sensory defects like rancidity and bitterness in cheese (Ma *et al*., 2000). In summary, vacuum level, except when abnormally high and thus deleterious also for teat integrity, mammary health and animal welfare, has mainly mechanical effects on milk extraction but does not affect milk composition directly.

3.4.2 Pulsation system

As discussed above, the pulsator has two main settings: the frequency of pulsations per minute and the time ratio between the sucking and massaging phases within a pulsation cycle. Increasing the pulsation ratio is equivalent to increasing the vacuum level and while efficient for rapid milking, an excessively high ratio (>66%) reduces milk flow by causing teat congestion, as with an excessively high vacuum level.

In contrast, increasing the pulsation frequency seems to increase milk flow (Clough & Dodd, 1956 for cows; Casu & Carta, 1974 and Le Du, 1985 for ewes; Lu *et al*., 1991 and Sinapis *et al*., 2000 for goats). This higher milk flow could partly be explained by the beneficial effect of compressive loads on the teat walls during the massaging phases, therefore benefiting the teat end by allowing it to return to its normal thickness much sooner after milking. Also, the higher milk flow could be explained by a higher intramammary pressure due to better stimulation of the animal and thus enhanced oxytocin secretion that expels the milk from the alveoli with great force (Marnet *et al*., 1996). We have to remember that milk quality changes throughout the process of milk ejection from the mammary gland and consequently milk is known to increase in fat content between the first milked spurts and the stripped milk at the end of milking (Guinard-Flament *et al*., 2001). This is because fat globules with diameters exceeding the smallest ducts are mechanically retained in the alveoli and need to be forcibly extracted by means of peri-alveolar myoepithelial cell contractions. This contraction is mainly the result of oxytocin action, so oxytocin injections significantly increase mean fat globule diameter (by 0.22 μm, from 4.25 to 4.47 μm) (Guinard-Flament *et al*., 2001).

In conclusion, because of this stimulating effect of pulsation frequency on milk ejection, Guinard-Flament *et al*. (2001) reported a significant increase in milk fat concentration. In contrast, protein content is not significantly affected because milk caseins and whey proteins are of limited size by comparison with fat globules and are uniformly drained throughout the ducts. It will be noticed that with the higher pulsation frequency used for goats and ewes, even the best electronic pulsators have difficulties in translating pulsation ratio information to the liner wall. In consequence, when a pulsator setting exceeds 90 pulsations/min, only a 50% ratio is observed on farms.

3.4.3 Mechanical effect of machine milking on milk quality

In addition to physiologically stimulating or inhibiting milk ejection and possibly increasing the risk of inflammation/infection at the teat level, many modern milking systems can modify milk quality by mechanical means due to air admission to the milking system, height of milk lines, pumping operation and cold storage in the bulk tank.

The main effect is seen on milk fat, which becomes more sensitive to external agents (lipolytic enzymes and oxygen) after impairment of the fat globule membrane. This allows easier contact between the triglycerides and lipases and increases the release of free fatty acids (FFA), especially butyric acid, and results in an increasing risk of taste defects and rancid odors (provoked lipolysis).

Mechanical turbulence due to mixing and bubbling of air and milk during transport, especially with high milk lines, shaking and forcing of milk into milk line elbows and fittings, abnormal air entry (air leaks), descent of milk from the line into the bulk tank and shaking of overly limited milk volume in the bulk tank, is found on 79% of farms with high FFA levels in milk. Flow in a milk line is complex because milk and air have to circulate together. When fat globules are totally submerged in the liquid phase of milk, the mechanical constraints on the fat globule wall are symmetrical. Conversely, when fat globules reach the milk–air interface, the mechanical constraints become asymmetrical and the fat globules become distorted. This

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mechanism is increased when fat globules encounter the wall of the milk line, subjecting the fat globule wall to shear stress and breakage. The lipases fixed on caseins are then released. The walls and the fat content of the fat globule are then spread at the milk–air interface and all are mixed when bubbles burst or merge (Evers, 2004). Thermal shocks in tanks and pumping defects also affect fat globule membrane integrity and are found on 58% and 67%, respectively, of farms with high FFA levels in milk (Rasmussen *et al*., 2006).

Nevertheless, it must be remembered that all the different kinds of milk do not have the same sensitivity to lipolysis, and it is well described that milk with high fat content and large- diameter fat globules is more sensitive to lipolysis induced by pumping than milk removed from animals fed low-fat diets or supplemented with unsaturated fatty acids (Wiking *et al*., 2004, 2005).

It is clear that some of these machine factors that contribute to excessive FFA development in milk can be eliminated by proper design, installation, maintenance and operation and/or by a better milking technique of the milker.

3.4.3.1 Specific action of cluster and liners

The choice of cluster and liners is very important for good milking efficiency. This element has to be considered as a whole, taking into account teat anatomy, milk flow of the animal, and the vacuum and pulsation setting used.

First, the total weight of the cluster is an element of choice. The more the cluster weighs, the higher the vacuum needed, with an increasing risk of vacuum fluctuations, liner slippage and air entry, leading to negative effects on teat condition. The weight can be mainly at the teat cup level or at the claw level, although a heavy claw requires a very well equilibrated udder to avoid excessive weight on the front teat. High vacuum and heavy clusters with adapted liners are sometimes recommended because of the associated shortened duration of milking but generally the tendency is now to simultaneously reduce cluster weight and vacuum level consistent with the long-term increased risk of negative effects on the teats.

Claw design and volume are important for vacuum stability under the teats. The kinetics of the milk while traveling from teat to long milk tube is also important in order to avoid the claw becoming clogged and milk extraction impeded. Additionally, there should be minimum air entry at the claw or at the base of the teat cup liner in order to maintain maximal milk transport. Verification of the cleanliness of the air entry hole is essential to avoid hydraulic milking and clogging of the claw with consequent vacuum fluctuations. However, it is also essential to avoid any recalibration of the holes that could lead to excessive air consumption at the parlor level consistent with the air reserve of the installation. Except for the risk of insufficient milk extraction and of milk impaction or hydraulic milking that could wash the teat end with contaminated milk, the impact on milk quality is low.

In the specific case of AMS, air entries are supplied at a higher level than in classical milking machines. The reasons are the lack of claw, individual longer short milk tubes and milk flow recorder per udder quarter, high number of valves to stop milking and transfer of dirty first milk spurts or colostrum to specific lines, and long milk line to the tank. The air to milk ratio can then be very high (8–10 : 1) for robots in comparison with a traditional installation (3 : 1) (Ipema & Schuiling, 1992), with a higher negative impact on lipolysis. Nevertheless, it needs to be stated that the latest generation of AMS tends to minimize this problem and such a system avoids all the problems of impaction and cross-contamination between teats.

Variations in the diameter of the short and long tubes and of claw volume are important elements controlling vacuum level and stability under the teat. Vacuum fluctuations increase with increasing milk flow, although variation up to 10 kPa will be uncommon under a milk flow of 7 kg/min (Goff & Leonard, 1978) that is also uncommon even with high-yielding cows. Nevertheless, increasing the bore of the short milk tube above the recommended diameter or a claw volume above 150 mL does not improve milking efficiency (O'Callaghan & Gleeson, 2000) and minimizes the effect of vacuum fluctuation due to these parameters on teat condition.

Whatever the brand of classical cluster, they increase lipolysis from 0.1 to 0.2 mEq/100 g milk fat, mainly due to air entry in the claw. Thus, excessive air entry at the claw level of 20 L/min can increase lipolysis by 0.43 mEq/100 g of milk fat compared with a normal air entry of 4–5 L/min (low range of the ISO standards NF ISO 5707). An air entry of 13 L/min at the claw level seems able to increase lipolysis significantly compared with an air entry of 6 L/min (O'Brien *et al*., 1998; Evers & Palfreyman, 2001).

This increase is more important when clusters have a periodical pulsator-controlled air inlet (BIO MILKER® type) or a continuous air entry inlet at the base of each teat cup or at the beginning of the short milk tube to maximize transport of milk to the claw and long milk tubes. As reported before for robotic systems without claws, such systems also lead to excessive air flow (ISO regulation) compared with a single air entry to the claw. Simple air leaks throughout the seal or shut-off valve at the claw level can additionally exceed 12 L/min and then increase lipolysis by 200%.

The results of many comparative experiments indicate that liner design has a greater effect on milking characteristics than any other machine factor (O'Shea *et al*., 1983). The

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effect is sensitive to strip yield (and thus milk quality), teat cup slip (mastitis risk), and milking time. The interaction with milking time is linked to the rigidity of the liner walls, estimated by the buckling pressure of the barrel. When rubber or silicone liner walls are thick or old, milk-derived products such as butterfat, phosphate and calcium deposits are absorbed onto and into the wall (Boast *et al*., 2008). This leads to increased buckling pressure, and liners need higher vacuum to collapse. Because the vacuum level is not adapted to liner age, the barrel walls have increasing difficulty in closing, which has the same effect as a shorter massage time. The risk for the teat end and teat sanitary status is thus higher.

Liner design is also very important (tubular or conical or even triangular, different barrel diameters, smooth or rigid mouthpiece). A narrow-bore liner barrel seems better for the teat than a wide-bore one, and an internal diameter 1 mm smaller than the average diameter of the teats before milking is recommended. This reduces edema and congestion of teats submitted to high vacuum levels of 50 kPa and/or high pulsator ratio (67 : 33) (Gleeson *et al*., 2004). In all cases, the effect of the liner is mainly physical via relatively good translation of pulsation parameters to the teats, with relatively unimportant effects on milk quality.

3.4.3.2 Specific action at the milk pump level

Continuous pumping of milk increases lipolysis (O'Brien *et al*., 1998). The temperature of milk is also a risk factor for lipolysis, and pumping milk at 31°C rather than 4°C significantly increases lipolysis (Wiking *et al*., 2005). The most common milk pumps are centrifugal and the rotation speed might have a proportional negative impact on lipolysis (Escobar & Bradley, 1990). In the same way, underfeeding of the milk pump is another risk factor. When the milk level detector in the receiver, which is used to operate the control circuit of the pump, is not stable, or when there is a lot of foam in the receiver that can mislead the level detector, the milk pump can remain in action until the receiver is empty, leading to aspiration of a mixture of air and milk and producing milk foam. The increase in lipolysis is then three to four times higher than with settled milk (Table 3.1).

Table 3.1. Lipolysis and milk pump function.

Milk pump function	Lipolysis (mEq/100 g of fat after 24 hours at 4°C)		Rate of increase of lipolysis (%)
	Receiver	Pump outlet	
Normal	1.41	1.58	12.1
With air aspiration	1.37	1.95	42.3

Source: Institut de l'élevage (2009), reproduced with permission of La France Agricole edition.

3.4.4 Optional components

Electronic milk meters (milk measurement devices) are now widely used by farmers as a tool for managing herd/flock performance. Most developed countries operate a national milk records program that implies measurement of milk yield and, after proportional sampling throughout milking, of fat, protein, SCC, and sometimes lactose from individual animals at regular intervals throughout the lactation. Because of the development of new tools for genomic selection, many other phenotypic criteria are now tested and these will undoubtedly be incorporated into milk meters so that they become increasingly complex multicriteria measurement tools (milk flow, online SCC, blood detector, hormonal probe, near infrared spectrometry for milk fat). In this respect, a large majority of on-farm milk meters sold are now certified by the International Committee for Animal Recording as devices that can be used as official portable milk meters for performance measurement (verification of accuracy over a large range of flow rates, low sensitivity to foaming, good reliability over widespread farm and milk compositions, and efficiency of sampling device). Milk meters incorporate many methods of measurement, but the most frequent electronic device uses a volumetric method. Milk flows into a measuring chamber and as soon as the quantity of milk in the chamber reaches a particular volume, usually 100 or 200 g, an outlet valve is opened and the inlet valve is closed. Each cycle of filling/emptying is counted. Milk meters can also measure the weight of milk in the chamber by way of a strain gauge. Some of these milk meters are designed to measure proportionate samples of the milk and not the full yield but their accuracy is generally limited to a narrower range of flow rates. Conversely, the latest devices are mostly flow meters rather than milk meters. They measure flow rate to empty a chamber or real-time flow rate by means of electrodes or infrared technology.

There is no clear effect of milk metering on milk quality even if milking conditions are obviously modified, depending on the technology and the complex kinetics and circulation of milk through the milk meter. An increase in lipolysis of 0.15 mEq/100 g fat with the use of milk meters has been reported, but with very low deviation between extremes (0.08 mEq/100 g fat) (Institut de l'élevage, 2009).

Another option is automatic cluster removal (ACR). Widespread in new cow parlors and even adapted for milking systems in barns, automatic take-off systems are

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generally coupled to milk meters that use milk flow measurements to decide the best moment to stop vacuum and retract the cluster (generally between 200 and 400 mL/min for a cow). In small ruminants, because of the price of such equipment and of the high number of places in the parlor due to size of the herds, ACR systems are mainly time regulated. This requires very homogeneous herds to avoid under-milking (milk left in the udder) in high-yielding or long-lasting milking animals or, conversely, to limit over-milking of low-yielding animals or those with a high milk flow rate.

When stable, there is no effect of ACR on milk quality if the volume retained in the udder is not too high (100–200 mL per quarter in cows). The main objective of such devices is to limit over-milking. This first allows reduction in time spent on the machine and shortens milking, especially when the number of milkers is low in relation to the number of animals in the parlor. It has also been proposed that ACR reduces the risk of mastitis but effects on udder health are not marked even if over-milking in conjunction with vacuum fluctuations could increase the risk of new infection (Rasmussen, 2004).

3.4.5 Milking parlors and milking stalls

Around the world, one can find all kinds of milking machines and milking parlors. The simplest autonomous milking machine comprises a vacuum pump and single or dual buckets for milking one or two animals simultaneously, widely used on small farms with only a few animals to milk. When the number of animals increases and/or when the milker wants to milk in a stanchion barn, a more powerful vacuum pump able to be connected simultaneously with several buckets can be used. Nevertheless, if milk production is important and the weight of full buckets becomes limiting, then it is best to connect the clusters directly to a milk line, which is widely used in northern Europe and America with high-yielding animals in tie stall barns as well as in outdoor mobile milking parlors. When time is limited because of increased numbers of animals, it becomes necessary to milk in specific parlors where animal and human circulation is separated. The smallest parlors have two to ten places, sometimes with parallel positioning of the animals ("tunnel" or side-opening "tandem" systems) (Fig. 3.6 and see Plate 3.4). Middle-sized parlors and most common installations keep animals standing on an elevated platform in a 30–70° angled fashion called a "herringbone" that faces away from the operator area. The biggest linear installations need the animals angled perpendicular to the pit and are called "side-by-side" parlors (Fig. 3.7 and see Plate 3.5). When the number of animals exceeds 200–400 (obligatory for over 1000 animals) and/or the number of milkers is limited, it is better to use rotary or turnstile parlors that can require only three operators (Fig. 3.8 and see



Figure 3.6. Tandem milking parlor. For a color version of this figure, see Plate 3.4.

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(a)



(b)

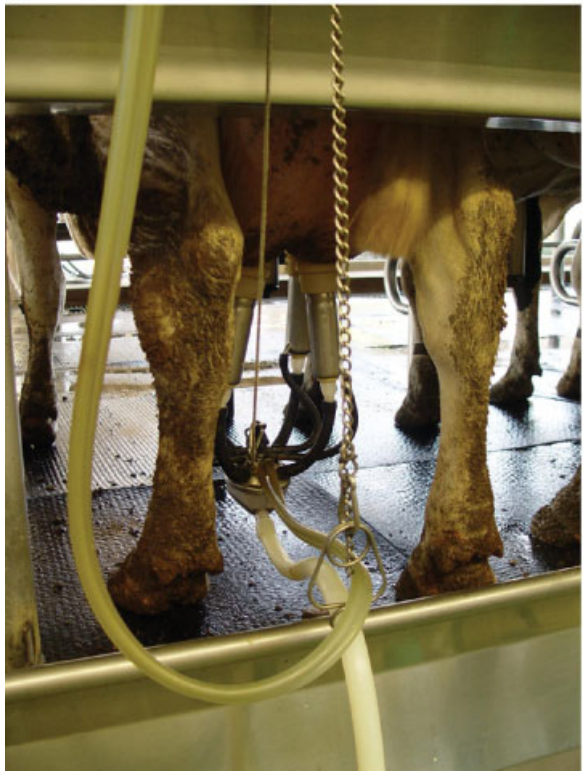


Figure 3.7. (a) Side-by-side milking parlor with rapid exit; (b) view of a cow in a side-by-side milking parlor. Access to the mammary gland is limited and observation of the cow reduced. Courtesy of Dairymaster. For a color version of these images, see Plate 3.5.

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Figure 3.8. Rotary parlor milking system. For a color version of this figure, see Plate 3.6.

Plate 3.6). In countries with higher workforce costs (Europe, Japan) and generally limited herd size (50–200), AMS were implemented about 20 years ago to reduce labor associated with milking and are now widespread (over 15% of installations in European countries). Mobile AMS for pasture have also been developed. Because the number of animals continues to increase, farmers are obliged to increase the number of AMS, generally one for every 50–60 cows, and this becomes very expensive. In 2010, the first AMS adaptable on a rotary platform was developed (AMR™ manufactured by De Laval).

There is no obvious direct relationship between milking parlor type and size or equipment and milk quality. Milk can be very clean and rich when collected in old herringbone parlors, and dirty and of poor quality when collected in new rotary parlors with all the modern automatisms.

The change from the classical bucket milking and carrying pails, to bucket milking with 140-L containers on wheels for transferring milk from cows to bulk cooling tanks, to pipeline installations (in barn or parlor) has been well reported to increase milk lipolysis (Pillay *et al*., 1980; Jellema *et al*., 1986) as a result of increased milk shaking, bubbling and foaming during transport between animals and storage tank. An increase in milk bacteriological quality due to reduced milk manipulation, speed of collection and cooling and hygienic methods used is also evident.

Different pipeline systems also have diverse effects. When the pipeline is high (over 2 m) and the milk requires vertical elevation, and when the line is also long with numerous elbows and fittings as often found in barn pipelines, the risk of lipolysis increases. It is well known that high milk lines increase the FFA content of milk (Gilson & Cousins, 1985; Heuchel & Chilliard, 1988; O'Brien *et al*., 1998 in cows; Morand-Fehr *et al*., 1983 in goats). However, some authors have recorded only a few differences in ewe milk with intermediate lines, while emphasizing the effect of delivery line design from receiver to bulk tank and especially the deleterious effect of vertical sections (Diaz *et al*., 2004). Another important source of mixing is the height the milk drops from the exit of the milk line into the tank. This problem can be avoided by connecting the milk line directly to the bottom outlet of the tank or tangentially to the tank wall.

Thus except for problems of poor installation, at the parlor level one of the remaining problems is the workforce and the adaptation of milking methods to parlor capacity consistent with the milking rate expected while incorporating observation time, washing and stimulating animals, dipping teats, and problems of under- or over-milking.

The introduction of AMS has initiated many studies on milk quality, especially because of the numerous differences with classical milking systems (rhythms of milking and fragmented milk collection, lack of claw, numerous

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pneumatic and/or electronic gates and valves, captors, etc.). When analyzing the modification of milk due to increased milking frequency (higher milk production, lower milk richness, lower SCC when the system is efficient, stable and maintained), the main effect is a systematic increase in lipolysis (Table 3.2) and some problems of teat cleaning leading mainly to high concentrations of spores of butyric acid bacteria. This is one of the reasons why some cheese of protected appellation of origin (PAO), such as Comté or Gruyère in France and Switzerland, have forbidden the use of AMS for their farmers (risk of rupture of cheese rind by gas development and of rancid taste; Table 3.3).

In a Dutch study performed on 28 dairy farms, switching from a classical milking system to AMS increased lipolysis without modification of SCC (Klungel *et al*., 2000), proving that AMS does not aggravate the mammary gland when correctly installed and maintained and after good animal adaptation. Nevertheless, lipolysis with AMS exceeded lipolysis in a classical milking system where cows were submitted to the same rhythm of three daily milkings, suggesting a specific effect of AMS. Conversely, Abeni *et al*. (2005) and Slaghuis *et al*. (2004) did not find any difference between classical milking systems and AMS when using the same milking rhythm. This is an example of the difference one can find between farms, installations and AMS technologies and possible effects of milk line and cooling device used (see following section). The conclusion is that there is no evidence that AMS induces significant deterioration of milk quality compared with classical installations.

Table 3.2. Free fatty acid content (mEq/100 g of milk fat) in milk from classical or automatic milking systems (AMS).

AMS	Classical milking system	Reference
1.13	0.86	Justesen & Rasmussen (2000)
0.53	0.38	Klungel *et al*. (2000)
0.72	0.51	Abeni *et al*. (2005)

Source: reproduced from Marnet & Billon (2009), with permission of Elsevier.

Table 3.3. Butyric acid (C4) release in milk from a classical or robotic milking system (RMS).

Text not available in this digital edition.

3.4.6 Storing and cooling devices

The storage of milk is not really considered a part of the milking procedure but a poor choice of materials and design could cancel all the benefits of a good milking system. During the cooling process, milk fat is subjected to fractionated crystallization with shrinkage of the globules and liquid fat expulsion. Additionally, in an accelerated creaming, this release increases the access of lipase, always efficient around 0°C, to fatty acids. However, storage of milk at low temperature in good conditions (effective regulation of the refrigeration system of the bulk tank, rapid drop of milk temperature to 4°C in less than 2 hours, refrigeration capacities adapted to volume received and frequency of milk collection, initiation of refrigeration only once the rotary agitator blades are submerged) does not affect lipolysis (Jellema, 1986) and does not change fat globule characteristics at less than 48 hours of storage (Couvreur *et al*., 2005). Nevertheless, the milk is necessarily subjected to repeated temperature fluctuations due to the different milk arrivals in the bulk tank. Even if they seldom exceed 15°C, these repeated rises in temperature can significantly increase the FFA content of milk (Cartier & Chilliard, 1989).

Freezing of milk when it comes into contact with the cold walls of the bulk tank is more problematic because the fat globule membrane may be disrupted by crystallization. On farms with excessive lipolysis due to cold shock of milk, the use of pre-cooling systems such as plate heat exchangers or tube coolers to cool milk prior to entry into the pumping system and storage tank can help solve these problems. This reduces damage to the fat globules, decreases the refrigeration power of bulk tanks, and thus also reduces lipolysis (Wiking & Nielsen, 2007). Nevertheless, the use of such pre-cooling systems is not a panacea against lipolysis because these systems can pose other problems (e.g., good cleaning).

In AMS, where milk arrives in very limited quantities (cow by cow) and at various intervals, the freezing risk of milk is very high and so the use of specific ice-cooled bulk tanks (expensive) or an additional small buffer tank of 300 L as a pre-cooling system is generally proposed. However, it must be remembered that lipase of bacterial origin, especially from psychrophilic bacteria that can develop even when milk is below 7°C, when they exceed 1 million organisms/mL, is very efficient and able to impair

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milk taste and odor. This makes the need for good hygienic milking practices before teat-cup attachment very obvious.

3.4.7 Cleaning systems

The manual flush cleaning of bucket and cluster is less and less used because it is time-consuming, labor-intensive, and the handling of chemicals and hot water is a potential hazard for milkers. The jetter system, also called cleaning-in-place, generally uses a third line plumbed to drums containing the cleaning solutions. An additional air injector is often necessary to ensure better cleaning because of the turbulence it creates in the milk line. This system based on constant recirculation of solutions in the installation is more economic with regard to water and chemicals and provides consistent cleaning every time. To make the washing procedure easy, the cleaning system can be fully automated once the cluster is attached to the jetters. The correct amount of chemicals is then added each time and the water temperature is regularly controlled.

This form of cleaning is divided into three cycles: rinse to remove milk residues after milking, wash to remove any remaining soil, and disinfect to kill bacteria remaining in the cleaned equipment. The milking system should be cleaned immediately after milking while the equipment is warm and before milk deposits start to form on pipes. The milk pipe is disconnected from the bulk tank. When milking is completed, any milk in the receiver vessel and the milk pump should be drained. The external surfaces of clusters and milking units are rinsed clean and the facility set up for the washing routine. This consists primarily of attaching jetters to the clusters and then transferring vacuum to the wash lines so that wash solutions are drawn into the wash lines, through the cluster and back to the recirculation.

An efficient wash routine requires good-quality water that has low bacterial count and low hardness, an adequate volume of solution to wash all milking plant surfaces, correct detergent strength, correct temperature of solution, sufficient contact time between detergent solutions and plant surfaces, adequate velocity of detergent solutions over milk contact surfaces, and sufficient slope of milk line for good water drainage at the end of cycles, which will remove milk residues and bacteria from the equipment. This will maintain milk quality, improve the appearance of the parlor, and prolong the life of the milking equipment.

The standard plate count of microorganisms has little diagnostic value in determining the source of bacterial contamination. A better indicator to understand the efficacy of cleaning systems is the presence of thermoduric bacteria. They survive pasteurization conditions and grow and multiply in the milking equipment, particularly old cracked rubber parts, if the cleaning and sanitation procedures are inadequate, especially with the use of cold water (Reinemann *et al*., 2003; Murphy & Boor, 2010). Recent work of Bava *et al*. (2009) showed that the maximum water temperature of the detergent phase had a significant effect on the coliform count of bulk tank milk. A temperature lower than 40°C during cleaning led to an increase in these bacteria and thus a minimum of 45°C and 10 min duration for the detergent phase is recommended (Reinemann *et al*., 2003). Indeed, milk contamination can also be the result of a poorly cleaned refrigerated bulk tank (MacKenzie, 1973; Thomas, 1974), which must be cleaned separately to the milking equipment.

If problems occur with the washing routine, then milk films will build up on the equipment. These films provide nutrients for bacteria, which can then multiply and increase their concentration in bulk milk even if the cows are in good health and milking hygiene is adapted. Because of its persistence and resistance, this biofilm becomes unaffected by the normal saline or detergent agents used. Proteolytic enzymes can sometimes be used in the sanitation process to remove this film due to their efficient action at low temperatures and concentrations, and because they degrade into environmentally friendly constituents(Pintaric, 2004).

Reverse-flow cleaning systems (or back-flushing) are those where water is pumped from the receiver through the milking machine and flushed back to exit at the cluster between each animal, limiting cross-contamination during milking. It is a very efficient system but expensive (an extra pumping system is needed) and consumes a lot of water. To prevent transfer of pathogens by the milking cluster, some AMS have a procedure for cluster flushing. The effect of cluster flushing on removal of pathogens was tested with cold water and with disinfectant and 98.4 and 98.9 %, respectively, of the pathogens were removed. Because cluster flushing can be performed with small amounts of water and without influencing the milking capacity of AMS, there are no reasons not to perform a simple cluster flush in milking systems (Schuiling, 2004).

Nevertheless, all cleaning procedures and especially cleaning agents can also damage the most sensitive parts of the milking equipment, such as rubber liners and tubes, as assessed by photomicrographs. However, silicone is more resistant to cleaning and sanitizing treatments. Thus, changes in the thermodynamics and morphology of milking surfaces caused by hygiene procedures have enormous importance in the adhesion of *Streptococcus agalactiae* and consequently in mastitis transmission between animals (Santos *et al*., 2011). It is important therefore that rubber parts are changed regularly according to the manufacturer's recommendation and number/duration of milking cycles, and not only when appreciated by sight or touch by the milker.

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3.4.8 New kinds of materials and sensing devices for better milk quality

AMS has seen development of many materials that aim to help the collection of better-quality milk and the animal breeder. The first developments concerned different sensing devices and associated software that detect and automatically discard milk of poor quality (decision threshold remaining the discretion of the milker). The main target was to detect mastitis, the method retaining the technology for measuring conductivity of milk from individual quarters combined with a check of milk volume of individual cows according to milking intervals for better accuracy (de Mol & Ouvweltjes, 2001). Nevertheless, the number of false alarms remains a problem and more sophisticated analyses including additional parameters (milk temperature, pH, Cl^- concentration) are in development and appear increasingly efficient (Ramirez Garcia *et al*., 2004). Another objective is to detect milk abnormalities. Optically based sensing devices are as good as visual inspection but remain limited. The best result (100% sensitivity, 99.6% specificity) was obtained with a green/blue/red LED array in detection of blood, colostrum and clots in milk (Whyte *et al*., 2004).

More recent developments include near-infrared analysis of SCC measurement at cow-side and for milk qualification. A noninvasive near-infrared analysis of udder tissue was also tested for *in vivo* mastitis diagnosis and may be developed in the near future with robotic systems using teat locations for optical scanning (Tsenkova *et al*., 2000, 2004). On the market already are direct cell counters for analyzing SCC at cow-side by measuring the DNA concentration of milk with automated and miniaturized methodologies similar to those used in reference laboratories.

Finally, integrated systems of data analysis have now been developed and commercialized to help in herd monitoring. They will be regularly enriched with other data easily recordable at the milking plant scale (milk flow characteristics, weight of animals, other specific milk quality parameters), and others are in development (biosensor for metabolic disease detection in expired air or to detect high-risk pathogens in milk such as *Salmonella* or *Listeria*).

3.5 MILKING PRACTICES

Pre-milking preparation is the time taken to manually clean and dry the teat surfaces before the milking machine is attached, which is essential for top-quality milk. This preparation, including teat massage just before teat-cup attachment, also provides effective stimulation of milk let-down and is essential for fast and complete milking.

Although today's high-producing Holstein cows require very little stimulation for normal milk let-down (Mein, 1995), manual stimulation for only 10 s is not adequate in late-lactation American Holsteins or European Friesians and Jersey cattle (Rasmussen *et al*., 1992), and a total pre-stimulation time of 10–20 s is not sufficient for most cows regardless of stage of lactation or milk production. This pre-milking preparation does not significantly lengthen total milking time and is even able to improve cow throughput (Reneau & Chastain, 1995). Pre-milking remains economically wise because of its ability to optimize the udder to produce better milk flow and improve milk quality.

Fore-stripping to check for clinical mastitis is another recommended pre-milking procedure but is physically tiring and labor-intensive. However, fore-stripping is a very powerful milk let-down stimulus and is therefore best used early during the cow preparation procedure. When minimal cow preparation (10 s) is being used, the addition of fore-stripping will ensure a consistent milk let-down response.

All these pre-stimulations induce oxytocin release. Udder massage (not only teat washing but vigorous udder floor manipulation) of 20–30 s is needed to induce an intramammary pressure rise equivalent to that measured after injection of a physiological dose (0.5 IU) of oxytocin (Labussière, 1999). This pressure needs to be at a maximum throughout the milking procedure to ensure the best extraction and to avoid elastic cisternal recoil of milk back to the alveoli as described by Caja *et al*. (2004). High-quality cow preparation will be required in herds using thrice-daily compared with twice-daily milking, because milk production and intramammary pressure are lower before milking. To reach the highest intramammary pressure a maximum interval of 2 min between stimulation and teat-cup attachment is required for high-yielding Holstein cows (Billon *et al*., 2006).

The preparation procedure also aims to improve milk quality. Studies show that good cleaning and drying with separate towels will reduce bacterial populations on teat surfaces by 75% (Galton *et al*., 1986). Teat sanitation by pre-dipping also reduces intramammary infection rate (Galton *et al*., 1988). Bacteria not removed from the teat surface before machine attachment will end up in the milk. There has been concern about psychrophilic bacteria and milk quality for some time. Psychrophilic bacteria thrive well at refrigeration temperatures, can survive pasteurization, and are the source of proteolytic enzymes or plasminogen activators that reduce dairy product shelf-life and yield (Ballou *et al*., 1995). Plasmin degrades milk casein even during cold storage and survives the high-temperature treatment of dairy processing. This is of great concern among dairy processors. *Salmonella* and *Listeria* are bacteria of human health concern. These organisms are also found in the cow's environment and can easily contaminate teat surfaces. Dairy managers need to find practical ways

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to include pre-milking teat sanitation into every milking routine in order to ensure milk quality and safety. During milking, stimulation has to remain important to ensure sustained oxytocin release, and for that optimized pulsation parameters are needed as previously reported.

After milking, when ACR is not used, the milker can proceed to stripping of the udder with the machine in place (machine stripping). Stripping allows the last fractions of milk blocked in the cisterns to be collected, but this has no real interest because the milk not extracted will be collected at the following milking. Thus, even if O'Shea (1987) reported an increase in mastitis rate with omitted machine stripping, this practice should be avoided if stripping milk does not exceed 15% of machine milk. If the stripping milk exceeds 15%, the machine is not adapted to the animal and/or the setting has to be revised to ensure better stimulation. On the other hand, stripping milk (or placing extra weight on the claw as frequently seen when vacuum level and liners are not adapted) dramatically increases the risk of new infection because of the risk of cup slippage and uncontrolled air entry, vacuum fluctuations, and increased risk of milk impaction.

At the end of milking, after cluster removal, cows and sometimes goats are submitted to post-dipping or spraying treatment with bacteriostatic products such as iodine or chlorhexidine. Sometimes, barrier products are also used. Despite these practices, microorganisms may enter the teat sphincter about 1 hour after milking because of the inability of the sphincter to close efficiently after keratin abrasion due to milk extraction, and sometimes to edema of the teat end often observed just after milking if vacuum was too elevated, massaging not sufficient, or after over-milking. Thus, it is recommended to hold the animals at the feed fences to stop them lying down in the stable or free stall cubicles after milking. At the next milking, washing of the udder is highly recommended to avoid residues of these products in the milk.

Under some exceptional conditions, milking can stress animals (unfamiliar or noisy surroundings, fear of foreign person or animals, acute pain due to poor function of milking machine, poor cluster manipulation or medical treatment in the parlor, health problems, and stray voltage sensitivity). These stressors are all able to inhibit oxytocin discharge (Bruckmaier *et al*., 1993) or limit oxytocin access to the mammary gland by dramatic reduction of blood flow in the udder (Gorewit & Aromando, 1985). The first consequence of such an inhibition of milk ejection is milk and milk fat retention in the alveoli (up to 75% of fat content in milk of dairy ewes) (Labussière, 1988). A second consequence is modification of milk composition. Cortisol and catecholamines produced in these stress conditions can be responsible for mobilization of fat reserves and release of long-chain (C18) FFA into blood. Milk composition can be modified as a consequence, as during the peak of lactation lipid mobilization is needed to cover milk component synthesis.

3.6 MILKING MANAGEMENT OF ANIMALS

To better adapt to a wider range of dairy systems worldwide, depending on breed and number of animals, food resources, easy access to technology and/or robotization, cost of manpower, etc., the milker tries to find the best equilibrium between workload (mainly because milking twice daily represents around 50% of the workload in a dairy herd or flock), milking time, milk quality and quantity and, more recently, acceptability by consumers. Such management choices are also able to modify milk quality and not only because of the equipment used.

3.6.1 Lowering milking frequency

This is of increasing interest for dairy farmers. Once-daily milking during a milking period (e.g., mainly at the end of lactation for cows in order to adapt milk collection to market needs or quota in Europe) or during all the lactation period (as often seen for goats) is a rapid and simple way to reduce working time or the need for extra staff without lowering milk production in the same proportion as time saved (high-yielding cows lose 30% of yield for 40% time saved, dairy goats lose 15% of yield for more than 23% of time saved; Komara *et al*., 2009).

Because milk secretion is physiologically downregulated to adapt to the maximum storage capacity of the udder, increasing the milking interval leads to a significant reduction in milk synthesis, generally after 20 hours in high-yielding ruminants. After a 24-hour interval, milk composition is also modified. Because lactose and proteins are the first constituents downregulated, reducing milk production in the same proportion, once-daily milking has significant effects on milk fat content (+4–5% in cows and ewes and +3 to –3% in goats depending on milk yield; Marnet & Komara, 2008). There is no evidence of other modifications of milk composition even after only one omission of milking per week (Meffe *et al*., 2003) compared with after once-daily milking (Chilliard *et al*., 2006; Komara *et al*., 2009). Nevertheless, at least in cows, less frequent milking is sometimes reported to increase the activity of plasminogen, plasmin, and plasminogen activator in milk. Changes in the activity of plasminogen and plasmin in milk have been positively correlated with increases in the concentrations of milk bovine serum albumin and plasma lactose, both of which are indicators of the disruption of tight junctions between mammary epithelial cells, indicating that paracellular leakage may have contributed to increased protease activity in milk during less

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frequent milking (Stelwagen *et al*., 1994). However, this modification, when observed, remains limited in comparison to that observed at the initiation of drying-off when mammary gland remodeling is induced by milk stasis (Aslam & Hurley, 1998), although it is never observed in goats where the large cisternal storage allows very long milking intervals (Komara *et al*., 2009). However, a reduction in milk lipolysis is reported by the cited authors without clear explanation.

3.6.2 Increasing milking frequency (three milkings and more per day)

This milking management system is not common, except in North America or China or in countries using AMS in which cows normally choose to be milked around 2.3–2.7 times per day. Generally, increasing milking frequency increases milk production (+10–25%), reduces milk fat content (–0.3 to 1%), and increases lipolysis, contrary to lowering milking frequency as shown in Table 3.4. Development of rancid odor and taste of milk products is a possibility because it is closely linked to the richness of C4, C12, C14 and C18:1 FFA, and a threshold concentration of 17.6 µg/mL of C4 in milk (Duncan & Christen, 1991) or 3 µg/g of C4 in butter (Urbach *et al*., 1972) are sufficient to impart a rancid taste and odor.

High-frequency milking is associated with an increase in acetyl-CoA carboxylase and fatty acid synthetase activity in the epithelial cells of goats (Travers & Barber, 1993). That could explain the *de novo* increase in short-chain fatty acid synthesis and higher lipolysis recorded in milk from high-frequency milked animals because lipase has more affinity for triglycerides with short-chain fatty acids (Klei *et al*., 1997). Additionally, large fat globules are also richer in short-chain fatty acids (Fauquant *et al*., 2005) and structurally less stable and could contribute to the higher FFA content even without any mechanical action (spontaneous lipolysis). However, frequent milking can also reduce SCC and improve mammary gland capacity to resist infection in addition to improving milk production efficiency (Dahl *et al*., 2004).

Table 3.4. Effect of milking frequency on the free fatty acid content (mEq/100 g of milk fat) in milk.

Twice daily	Three times daily	Four times daily	Reference
0.42	0.49		Klungel *et al*. (2000)[3]
1.14		1.49	Wiking *et al*. (2006)[1]
0.72	0.99		Klei *et al*. (1997)[2]
0.42	0.71		Slaghuis *et al*. (2004)[3]
0.33	0.44		Jellema (1986)[3]

Method of analysis: [1]autoanalyzer; [2]copper soap; [3]BDI "Bureau of Dairy Industries" detergent procedure.

3.7 CONCLUSIONS

Machine milking systems are complex, with many parts and parameters to control in order to ensure good animal stimulation, a smooth mechanical action for milking, and good conservation of milk. They also need to be regularly cleaned to ensure superior microbiological conditions and conservation of milk, and they need to be correctly and regularly adjusted and have wearing parts replaced in order to function sustainably and provide improved dairy food security. This remains an essential lever for reducing antimicrobial drug use in dairy herd management of mastitis. Thus the impact on milk quality is important and can rapidly depreciate all the efforts made at the level of the animal (genetics, food supply) and the workforce (animal care, time-consuming milking practices), making machine milking the second more important point for controlling milk quality and farmer benefits. Evolution of machine milking also needs to adapt equipment and settings to new human practices and management of dairy herds and flocks, especially at a time when the workload has became crucial to ensuring the sustainability of dairy systems across generations of farmers. Fortunately, the increasing automation and software control of these milking systems, and the ensuing integrated analysis of complex data, open a wide range of new tools for dairy management and the control of milker efficiency. It is a new era of the agriculture of precision and a great hope to ensure the continued functioning of dairy farms in the future.

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4
Milk Lipids

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4.1 INTRODUCTION

Milk lipids are a major source of energy for the newborn animal and human. They occur as fat droplets, which contain mainly non-polar lipids surrounded by a polar milk lipid globule membrane, with the droplets dispersed in the aqueous milk serum. The total lipid content of cow milk is in the range of 3–5% but the content depends on a wide variety of factors, including species of animal, breed, diet, stage of lactation and health of the animal. Milk fat includes compounds with a wide range of chemical structures that are classified as lipids due to their solubility in non-polar organic solvents and their low solubility in water. The main lipids present are the triacylglycerols, which comprise more than 98% of bovine milk lipids, although the content of triacylglycerols may vary according to species and quality of milk (Table 4.1). Milk fat acts as a carrier for the fat-soluble vitamins A, D and E, as well as β-carotene, which acts as provitamin A. These are discussed in detail in Chapter 10. Flavour compounds are also present in the lipid phase and these are discussed in Chapter 15.

4.2 FATTY ACIDS

The fatty acids are present in triacylglycerols, diacylglycerols and monoacylglycerols and phospholipids, and determine many of the lipid properties including nutritional effects. Small concentrations of free fatty acids also occur in fresh milk, but this concentration can increase during milk storage due to enzymic hydrolysis by milk lipoprotein lipase or bacterial lipase. Milk lipoprotein lipase is capable of catalysing the hydrolysis of both triacylglycerols and phospholipids to liberate free fatty acids in the presence of suitable co-factors (Stocks & Galton, 1980). No fatty acid specificity is shown by the enzyme, but it does exhibit strong positional specificity with *sn*-2 monoacylglycerols resistant to hydrolysis unless they isomerise to *sn*-1 or *sn*-3 isomers (Nilsson-Ehle *et al*., 1973). Rapid chilling of the milk reduces the rate of lipolysis, and the enzymes can be inactivated by heat treatment. Biosynthesis of fatty acids occurs *de novo* in the mammary gland, and lipids from the animal feed also provide a source of fatty acids that can undergo chain elongation or desaturation. Isomerisation and biohydrogenation of unsaturated fatty acids occur in the rumen of the cow and other ruminants, and this allows the formation of *trans*-unsaturated fatty acids and increased concentrations of saturated fatty acids. Approximately 400 fatty acids occur in bovine milk fat, although many are present at very low concentrations. The fatty acid composition of milk varies with season (Table 4.2), and depends on the feed, stage of lactation, and breed of cow or breed of other mammalian species. The content of fatty acid C18:1 *c*-9 was higher when cows were fed on fresh pasture whereas the content of saturated fatty acids increased when cows were fed on a mixed grass/maize silage mixture (Elgersma *et al*., 2004). The saturated fatty acids comprise about 62–78% of milk fat on a weight per cent basis, but their concentration on a molar basis is higher because of the presence of low-molecular-weight saturated fatty acids (Table 4.3). This subject has been reviewed by Jensen (2002). There has been considerable interest in conjugated linoleic acids (CLA) in recent years because of their interesting effects in human nutrition. CLA consist of up to

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Table 4.1. Concentration of the main classes of lipids in bovine, mares', human, goats' and ewes' milk.

Lipid class	Per cent of total lipids in bovine milk[1]	Per cent of total lipids in mares' milk[2,3]	Per cent of total lipids in human milk[2,4]	Per cent of total lipids in goats' milk[5,6]	Per cent of total lipids in ewes' milk[5,6]
Triacylglycerol	98.3	79.3	98	97.32	98.11
Diacylglycerol (DAG)	0.3	1.8 (including MAG)	n.d.	1.89 (including cholesterol, FFA)	1.45 (including cholesterol, FFA)
Monoacylglycerol (MAG)	0.03	—	n.d.	0.1	0.03
Free fatty acids (FFA)	0.1	9.4	Trace	(with DAG)	(with DAG)
Phospholipids	0.8	5	1.3	0.65	0.38
Unsaponifiables	1.5	4.5	0.7	n.d.	n.d.
Sterols	0.3	0.46	0.4–1.3	0.36	0.30

n.d., not determined.

Sources: based on data from [1]Walstra & Jenness (1984); [2]Malacarne *et al*. (2002); [3]Pikul & Wojtowski (2008); [4]Weaver & Prentice (2003); [5]Rodríguez-Alcalá & Fontecha (2010); [6]Park *et al*. (2007).

Table 4.2. Seasonal variations of the content of the main fatty acids in French cow butter (g FA/100 g butter).

	Winter ($N=18$)		Spring ($N=18$)		Summer ($N=18$)	
Fatty acid	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
C4:0	3.35	0.519	3.12	0.362	3.16	0.389
C6:0	2.35	0.35	2.2	0.25	2.16	0.277
C8:0	1.45	0.198	1.38	0.144	1.32	0.168
C10:0	3.25	0.353	3.15	0.302	2.9	0.336
C10:1	0.31	0.043	0.3	0.031	0.29	0.036
C12:0	3.62	0.25	3.58	0.257	3.2	0.27
C14:0	10.44	0.618	10.37	0.429	9.63	0.347
C15:0 *ante*+C14:1	1.07	0.088	1.06	0.064	1.09	0.069
C15:0 *iso*	0.41	0.046	0.45	0.049	0.5	0.037
C15:0	0.96	0.112	0.99	0.088	0.98	0.056
C16:0 *iso*	0.23	0.025	0.22	0.022	0.22	0.021
C16:0	26.24	1.968	24.98	2.267	22.27	2.049
C16:1	1.2	0.103	1.15	0.131	1.11	0.116
C17:0 *ante*	0.25	0.027	0.28	0.034	0.31	0.03
C17:0 *iso*	0.33	0.033	0.33	0.028	0.34	0.02
C17:0	0.42	0.049	0.41	0.036	0.44	0.041
C17:1	0.17	0.019	0.16	0.015	0.18	0.01
C18:0	6.41	0.459	6.91	0.764	7.48	0.635
C Trans 18:1	1.72	0.179	2.11	0.567	2.5	0.421
cis 18:1	12.57	0.611	13.2	0.791	14.34	0.933
18:2 (*n*-6)	1.06	0.076	1.07	0.088	0.97	0.124
18:2 (*c*-9, *t*-11)	0.4	0.058	0.52	0.169	0.74	0.162
18:3 (*n*-3)	0.26	0.081	0.35	0.111	0.43	0.088

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Table 4.3. Major fatty acids in bovine milk fat.

Fatty acid class	Carbon number*	Common name	Average range (wt%)[1]	Average range for Australian milk fat (mol%)[2]
Saturated	4:0	Butyric	2–5	9.8–10.8
	6:0	Caproic	1–5	4.0–4.5
	8:0	Caprylic	1–3	1.8–2.1
	10:0	Capric	2–4	2.9–3.6
	12:0	Lauric	2–5	2.8–3.5
	14:0	Myristic	8–14	8.6–10.1
	15:0	Pentadecanoic	1–2	n.d.
	16:0	Palmitic	22–35	18.4–20.3
	17:0	Margaric	0.5–1.5	n.d.
	18:0	Stearic	9–14	11.1–12.0
	Total saturates		62–78	n.d.
Monounsaturated	16:1	Palmitoleic	1–3	n.d.
	Total 18:1		20–30	19.9–23.2
	18:1 (*t*-11)	Vaccenic acid	0.4–4.0[3]	n.d.
Polyunsaturated fatty acids	18:2	Linoleic	1–3	n.d.
	18:2 (*c*-9, *t*-11)	Rumenic	n.d.	n.d.
	18:3	α-Linolenic	0.5–2	n.d.

*Fatty acid shorthand nomenclature indicates number of carbon atoms, followed by a colon, followed by number of double bonds (stereochemistry, i.e. *cis* or *trans* and position from carboxylic carbon atom). n.d., not determined.

Sources: based on data from [1] Kaylegian & Lindsay (1995); [2] Parodi (1979); [3] Collomb *et al*. (2006).

28 positional and geometric isomers of octadecadienoic acid with conjugated double bonds positioned at carbon numbers 6, 8–12, and 14, and with configurations *cis–cis*, *cis–trans*, *trans–cis*, or *trans–trans*. A range of CLAs are present at low concentrations in bovine milk, typically at 0.2–3.7% fat, although contents up to 5.4% have been reported (Collomb *et al*., 2006). Rumenic acid (C18:2 *c*-9, *t*-11) is the main CLA component, but a range of *cis–trans* or *trans–trans* CLA isomers with the *trans* bond at C-7 to C-14 and the *cis* bond at C-9 to C-13 is found. Vaccenic acid (C18:1 *t*-11) is the main monounsaturated *trans* fatty acid, being present at 46.5% of total *trans* fatty acids (O'Donnell-Megaro *et al*., 2011). Because of interest in the beneficial effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), attempts have been made to increase their concentrations by supplementing the diet of cows with these fatty acids, but the results have been mixed depending on feed type and animal species (Lock & Bauman, 2004). Experiments with lactating ewes fed flaxseed for example have shown a significant decrease in saturated fatty acids (C6:0 to C16:0) and a significant increase in C18:1 *t*-11 and C18:2 *c*-9, *t*-11 fatty acids in their milk (Caroprese *et al*., 2011).

4.3 TRIACYLGLYCEROLS

The triacylglycerols contain three fatty acid molecules esterified to glycerol (Fig. 4.1). With over 400 fatty acids

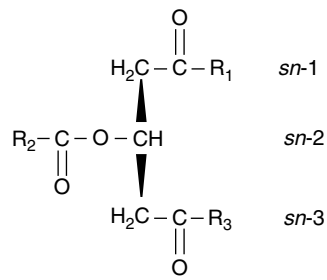


Figure 4.1. Fischer projection formula of a triacylglycerol.

present in triacylglycerols, the number of distinct molecular species is well over 100 000, although the fatty acid distribution is not completely random. Palmitic acid (C16:0) is esterified preferentially at the *sn*-1 and *sn*-2 positions, whilst stearic acid (C18:0) is mainly at the *sn*-1 position and oleic acid (C18:1) occurs mainly at the *sn*-1 and *sn*-3 positions. The short-chain fatty acids butyric (C4:0) and caproic (C6:0) acids are esterified mainly at the *sn*-3 position. Kalo *et al*. (2009) quantified about 450 of the main triacylglycerols in milk fat with carbon numbers between 12 and 57 and with 0-6 double bonds (Table 4.4). The relative concentrations of saturated, monoene, diene, triene, tetraene, pentaene and hexaene triacylglycerols have been

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Table 4.4. Carbon numbers of groups of triacyglycerols (TAGs) in bovine milk fat present at >1 mol%.

Carbon number	Concentration (mol %)	Main TAGs present
32:0	1.7114	
34:0	3.8773	16:0/14:0/4:0; 18:0/12:0/4:0
36:1	2.5690	
36:0	6.9363	16:0/16:0/4:0; 18:0/14:0/4:0
38:2	1.0394	
38:1	5.3858	18:1/16:0/4:0
38:0	4.7441	18:0/16:0/4:0; 18:0/14:0/6:0; 16:0/16:0/4:0
40:2	1.9585	
40:1	3.9709	18:1/18:0/4:0; 18:1/16:0/6:0; 18:0/16:1/6:0
40:0	3.2236	
42:1	2.6539	
42:0	2.8179	
44:1	2.3504	
44:0	2.6754	
46:1	3.0213	
46:0	3.1052	16:0/16:0/16:0; 18:0/14:0/16:0; 18:0/12:0/18:0
48:2	1.4481	
48:1	3.7729	
48:0	2.8485	
50:2	2.7974	
50:1	6.0234	18:1/16:0/16:0; 18:1/14:0/18:0; 16:0/18:0/16:1
50:0	2.4345	
52:3	1.2858	
52:2	4.2819	18:1/16:0/18:1; 18:2/18:0/16:0
52:1	3.2611	18:1/16:0/18:0; 18:0/16:1/18:0
52:0	1.1432	
54:3	1.6438	
54:2	1.6521	

Source: reproduced from Kalo *et al*. (2009), with permission of Springer Science+Business Media.

reported as 40.0, 38.4, 16.2, 4.5, 0.6, 0.1 and 0.03 mol%, respectively (Kalo *et al*., 2009).

4.4 POLAR LIPIDS: PHOSPHOLIPIDS AND CHOLESTEROL

A polar membrane (milk fat globule membrane, MFGM) encloses milk fat globules and allows them to be dispersed in milk as small droplets. The inner layer of the MFGM is formed in the endoplasmic reticulum, and it is surrounded by a bilayer rich in phospholipids, cholesterol and proteins derived from the mammary epithelial cells. Polar lipids from the MFGM become part of the fat phase of the milk and occur in a concentration of 9.4–35.5 mg per 100 g in raw milk (Rombaut & Dewettinck, 2006). This corresponds to a concentration of 0.2–1.2% in the milk fat. The main phospholipids are phosphatidylethanolamine (PE) (19.8–42% w/w), phosphatidylcholine (PC) (19.2–37.3% w/w), phosphatidylserine (PS) (1.9–10.5% w/w) and phosphatidylinositol (PI) (0.6–11.8% w/w), with sphingomyelin (18.4–34.1% w/w), phosphatidylglycerol, plamalogens, and traces of lyso-PC, lyso-PE, ceramides, diphosphatidylglycerol and gangliosides also present (Gallier *et al*., 2010) (Fig. 4.2). The fatty acids in the phospholipid fraction contain more polyunsaturated fatty acids and less saturated fatty acids than the triacylglycerol fraction, with 36.6% saturated, 24.9% monounsaturated and 38.5% polyunsaturated fatty acids being reported for the total phospholipid fraction and with contents varying in the different phospholipid classes (Table 4.5) (Gallier *et al*., 2010).

Cholesterol represents 95% of the total sterols in milk, with 10–20 mg/dL of cholesterol present in whole milk or 0.3–0.6% when expressed as a percentage of fat content (Jensen & Newberg, 1995). The cholesterol content is similar in goats', cows', camels', yaks' and human milk (Chilliard, 1997; Konuspayeva *et al*., 2008; He *et al*., 2011).

4.5 CONJUGATED LINOLEIC ACIDS

Animal experiments with CLA created a lot of interest by reporting that CLA had anti-carcinogenic and lipid-lowering effects and caused reduction of body fat (Terpstra, 2004). This focused attention on CLA as a dietary component. The daily intake of CLA has been estimated at 95–440 mg, but this intake varies widely from country to country and between individuals. However, vaccenic acid can be desaturated to *cis*-9, *trans*-11 CLA in the human body, with a mean conversion rate of 19% reported by Turpeinen *et al*. (2002), so endogenous synthesis increases the CLA available to tissues by a considerable amount above dietary intake (Collomb *et al*., 2006). In a study with Holstein and Brown Swiss cows, it was shown that the CLA concentration in milk fat and the CLA desaturase index varied over threefold between individuals, but breed differences were minor (Kelsey *et al*., 2003). The concentration of CLA in milk fat and the CLA desaturase index were essentially independent of milk yield, milk fat percent, and milk fat yield. However, seasonal effects on the concentration of CLA in milk fat have been reported, with the highest concentrations being reported for fat from cows that are consuming fresh grass due at least partly to an

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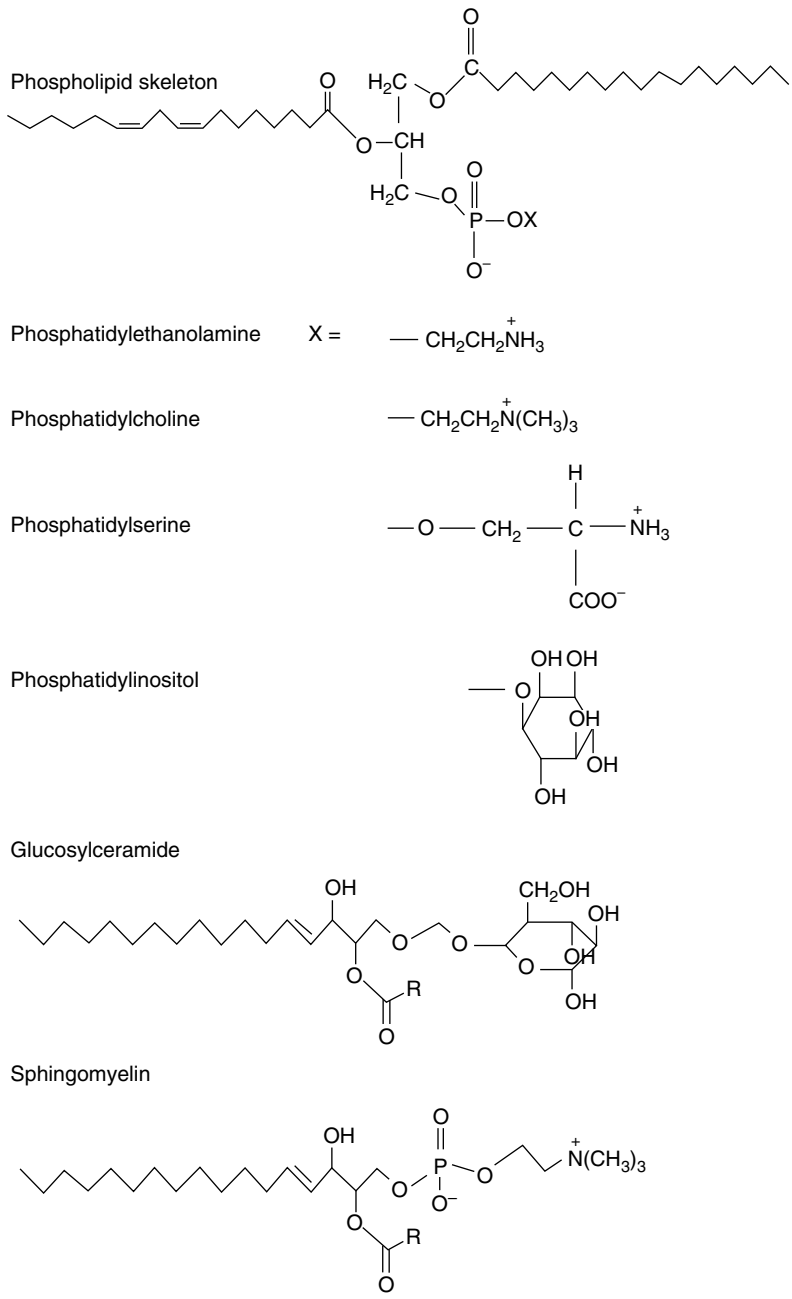


Figure 4.2. Structures of some polar lipids in milk.

increase in Δ^9-desaturase activity in the mammary gland (Lock & Garnsworthy, 2003). The effects of different plant oils on CLA content of milk fat have also been studied, with oils rich in linoleic acid being more effective than fats containing less polyunsaturated fatty acids in increasing CLA concentrations (Collomb *et al*., 2004), although dietary fish oils may be even more effective (AbuGhazaleh *et al*., 2001). The flavour stability of milk fat does not deteriorate with increased CLA content unless the increase in CLA content is very high. Thus the feeding of extruded soybeans or fish oil did not adversely affect the flavour or cause the flavour of pasteurised milk or butter to

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Table 4.5. Major fatty acids in bovine milk fat phospholipids.

Phospholipid	Total concentration (mol%)	Saturated fatty acids (mol%)	Monounsaturated fatty acids (mol%)	Polyunsaturated fatty acids (mol%)
lyso-PC	1	0.6	0.3	0.1
PC	36.6	11.7	11.5	13.3
SM	21.8	20.9	0.9	n.d.
ePC	5.4	1.3	3.4	0.7
lyso-PE	0.5	0.1	0.3	0.2
PE	22.6	0.1	4.5	18.1
PE-cer	0.0087	0.006	0.003	n.d.
ePE	1	0.0	0.3	0.6
PI	2.9	0.0	1.1	1.8
PS	1.7	0.0	0.7	1.0
PG	4.6	1.9	1.7	1.0
PA	1.8	0.0	0.1	1.6

lyso-PC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin; ePC, ether phosphatidylcholine; lyso-PE, lysophosphatidylethanolamine; PE, phosphatidylethanolamine; PE-cer, phosphoethanolamine-ceramide; ePE, ether phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid.

Source: based on data from Gallier *et al*. (2010).

deteriorate more rapidly than that of the control sample (Ramaswamy *et al*., 2001).

The different CLA isomers may have different physiological effects (Martin & Valeille, 2002). The *cis*-9, *trans*-11 CLA represents over 75% of the CLA in milk fat, whereas *trans*-10, *cis*-12 CLA is a minor isomer. However, in most human studies synthetic mixtures containing both isomers in comparable concentrations have been used. This has made it more difficult to draw useful conclusions from human studies. The *trans*-10, *cis*-12 CLA has been reported as the component responsible for decreasing plasma glucose concentrations and increasing insulin sensitivity but other studies have reported that this isomer increases insulin resistance (Khanal, 2004). Although animal studies suggested that CLA may lower plasma cholesterol and triacylglycerol concentrations, most human studies have found no significant effect on plasma total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations (Terpstra, 2004). CLA was also found to have no significant effect in humans on body weight or body weight regain after a weight loss programme. Studies by Blankson *et al*. (2000) and Thom *et al* (2001) reported that ingestion of capsules containing 3.4 and 1.8 g/day CLA caused significant reductions in body fat, but the studies were small with only 15 and 20 participants, respectively. Other studies have not reported any significant reduction of body fat (Terpstra, 2004). Animal studies have also shown that ingestion of CLA can reduce the incidence of tumours in rats treated with carcinogenic reagents (Park *et al*., 2004). Studies with human tumour cell lines have also shown the anti-proliferative effect of CLA (Maggiora *et al*., 2004). However, the evidence on the anti-carcinogenic effects of CLA is still not sufficiently convincing to allow a conclusion to be reached, with some reports even reporting pro-carcinogenic effects (Wahle *et al*., 2004). The effect of dose of CLA is poorly understood, but this may be an important variable in these studies.

4.6 GENETIC INFLUENCES ON MILK FAT CONCENTRATIONS AND FATTY ACID PROFILES

The fat content of milk varies quite widely between breeds of the same species, and there are also big differences between species. Values of 3.7 ± 0.32%, 5.42 ± 0.43%, 4.76 ± 0.44%, 4.12 ± 0.22%, 4.28 ± 0.39% and 3.58 ± 0.26% have been reported for Holstein, Jersey, Guernsey, Ayrshire, Brown Swiss and Milking Shorthorn cows, respectively (Cerbulis & Farrell, 1975). Reported values for the fat content of goat, sheep and yak milk are even higher than for Jersey cows (Table 4.6).

The fatty acid content among bovine breeds varies less widely than the fat content but a wide range of values has been reported for camels' milk from different areas of Jordan (Table 4.6). Goat milk differs from cows' milk in its composition, with higher concentrations of C6–C10 saturated fatty acids, but lower concentrations of C4, C16 and C18:1 (Chilliard, 1997).

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Table 4.6. Effect of breed and species on major fatty acids in milk fat.

	Bovine breed[1]							
	Dutch Friesian	Meuse-Rhine-Yssel	Groningen White Headed	Jersey	Camel[2]	Goats[3]	Sheep[4]	Chinese yak breeds[5]
Per cent fat	4.73	4.63	4.53	6.16	3.39*	6.25	7.03	6.51
Fatty acid (g/100 g of fat)								
C4:0	3.82	3.65	3.63	3.59	0.04–0.22	2.43	17.4†	—
C6:0	2.64	2.56	2.33	2.44	0.41–3.81	2.71	—	0.96
C8:0	1.59	1.52	1.32	1.39	0.14–2.44	2.95	—	0.66
C10:0	3.87	3.66	2.98	3.36	0.11–1.88	11.09	—	1.52
C12:0	4.74	4.75	3.69	4.05	0.7–3.42	5.26	20.1‡	1.57
C14:0	12.73	12.79	11.61	11.65	7.64–13.32	10.35	—	7.97
C16:0	30.9	29.54	28.98	33.64	18.16–32.48	32.57	33.2	26.58
C18:0	10.43	10.32	10.82	10.93	6.96–15.2	5.08	7.2	18.93
Total saturates	74.06	72.32	68.86	74.27	50.15–64.12	75.13	77.9	64.62
C18:1 (*c*-9)	18.39	19.47	21.98	18.25	16.64–32.88	15.11	11.8	21.78
Total *trans* C18:1	1.29	1.55	1.76	1.34	—	1.87§	1.98	5.77
C18:1 (*t*-11)	0.7	0.98	1.25	0.9	—	0.98	0.85	4.78
C18:2 (*n*-6)	1.62	1.41	1.16	1.08	0.17–3.13	1.69	3.16	1.12
C18:2 (*c*-9, *t*-11) (CLA)	0.3	0.41	0.57	0.31	0.06–1.56	0.685	0.35	0.92
C18:3 (*n*-3)	0.58	0.97	1.38	0.87	0.09–6.15	0.14	0.14	1.62
Total unsaturates	23.18	24.26	26.73	22.68	35.89–49.85	24.87	22.03	35.38

*Value from Haddad *et al*. (2010).

†Includes 4:0–11:0.

‡Includes 12:0–14:0.

§Does not include C18:1 (*t*-15) and C18:1 (*t*-16), which were not separated from *cis* isomers of C18:1.

Sources: based on data from [1]Maurice-Van Eijndhoven *et al*. (2011); [2]Ereifej *et al*. (2011); [3]Marin *et al*. (2011); [4]Abbeddou *et al*. (2011); [5]He *et al*. (2011).

The fatty acid composition of sheep milk has been found to vary for different breeds, with a correlation with milk yield and fat content. As the milk yield increased, total and short- and medium-chain saturated fatty acids were reduced, and the C18:0 and polyunsaturated fatty acid content increased (Signorelli *et al*., 2008).

Milk yield increases for a few weeks after parturition, but the fat content falls. The fat content of milk from Holstein heifers fell from 6.0% to 3.8% within 24 hours of calving (Nardone *et al*., 1997). Gross *et al*. (2011) have discussed the effects of stage of lactation on milk fat composition. They reported that changes in milk fat composition were most marked in the 6-week period after calving. Fatty acids up to C16:0 were low in colostrum, but increased progressively, whereas C18:1 *c*-9 decreased over a 12-week period. The concentration of polyunsaturated fatty acids remained relatively constant. These changes can be interpreted in terms of changes in the balance between fatty acids derived from the diet, *de-novo* synthesis in the mammary gland, rumen metabolism and body fat mobilisation (Stoop *et al*., 2009).

4.7 INFLUENCE OF FEEDS, FEEDING REGIMES, PASTURE AND STAGE OF LACTATION ON MILK LIPIDS AND THEIR LEVELS

Forages are often major sources of lipids in ruminant diets. The fat content and fatty acid composition of grasses available for consumption by ruminants are dependent on a range of environmental factors. These include effects of season, number of cuts, and fertiliser used. Significant losses of α-linolenic acid in forage can occur during haymaking or during wilting prior to ensiling. The concentrations of C18:3 *n*-3 and C18:2 *n*-6 in bovine milk were increased when cows were fed red clover silage compared

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with grass silage, but the concentration of each fatty acid remained below 2% (Dewhurst *et al*., 2003; Al-Mabruk *et al*., 2004). High polyphenol oxidase activity in red clover can lead to reduced biohydrogenation in the rumen, and a consequent increase in polyunsaturated fatty acids (Van Ranst *et al*., 2011). Grazing cows in pastures increases the concentration of polyunsaturated fatty acids in milk, with concentrations up to 3.2% C18:3 *n*-3 reported (Chilliard *et al*., 2001). This subject has been reviewed by Chilliard *et al*. (2001) and Dewhurst *et al*. (2006).

Including relatively high concentrations of starch in cattle feed tends to cause a reduction in milk fat yield, which is known as milk fat depression. This effect has been attributed to a reduction in the pH of the rumen in low-fibre diets (Bauman & Griinari, 2003). However, a reduction in milk fat yield was reported to occur following the inclusion of more fermentable starch without any corresponding effect on the pH of the rumen (Oba & Allen, 2003). Incorporation of dietary sugar or molasses in a high concentrate diet can help to reduce milk fat depression (Martel *et al*., 2011), although the incorporation of molasses is not always beneficial (Oelker *et al*., 2009). Protected CLA supplements caused reductions in milk fat yield and content compared with control, the reductions in yield averaging 34% for calcium salts of CLA and 44% for formaldehyde-treated CLA supplements (de Veth *et al*., 2005).

Higher concentrations of polyunsaturated fats can be incorporated into ruminant milk by the incorporation of plant or marine oils, vegetable oilseeds or rumen-protected or inert lipids in the diet. Dietary supplementation with flaxseed significantly increased the concentrations of monounsaturated fatty acids, conjugated linoleic acid, and polyunsaturated fatty acids in ewe milk (Caroprese *et al*., 2011). Based on a meta-analysis of trials involving the use of lipid supplements, Glasser *et al*. (2008) concluded that the greatest variation in milk fatty acid composition due to consumption of supplements was in the percentages of medium-chain versus C18 fatty acids. Among the C18 fatty acids, C18:0, *cis*-C18:1 and *trans*-C18:1 showed most variation. The percentages of C18:2 *c*-9, *c*-12 and C18:3 were elevated when protected lipids (mostly formaldehyde treated) were provided. Increases of 76% in C18:2 and 123% in C18:3 were reported when formaldehyde-treated canola seeds were fed to Holstein cows (Tymchuk *et al*., 1998). Goat and sheep milk is more susceptible to manipulation of composition by dietary changes than cows' milk (Sampelayo *et al*., 2007).

4.8 DIGESTION OF MILK FAT

About 10–30% of dietary fat is hydrolysed to 1,2-diacylgycerols and fatty acid by gastric lipase in the stomach. Fat then passes into the duodenum, where pancreatic lipase continues the hydrolysis of fat into 2-monoacylglycerols and fatty acids. These more polar lipids are incorporated into mixed micelles with cholesterol and fat-soluble vitamins. Small micelles are transferred across the enterocyte brush border membrane. Longer-chain fatty acids are transferred by intracellular fatty acid binding proteins within the enterocyte and re-esterified to form triacylglycerols, which are transported to the liver in the form of chylomicrons. Short- and medium-chain fatty acids pass through the mucosa of the stomach and are taken up directly into the portal vein. Chylomicrons and very low density lipoproteins (VLDL) formed in the liver are the main particles responsible for transporting triacylglycerols to the tissues. The smaller denser lipoproteins LDL and HDL are responsible for the transport of cholesterol to and from cells.

4.9 NUTRITIONAL EFFECTS OF MILK FATTY ACIDS

More people die annually worldwide from cardiovascular disease (CVD) than from any other cause. In 2008, an estimated 17.3 million people died from CVD, which comprised 30% of all deaths. Of these deaths, an estimated 7.3 million were due to coronary heart disease (CHD) and 6.2 million were due to stroke (World Health Organisation, 2011). Following work by Keys *et al*. in the 1950s, the hypothesis that dietary saturated fatty acids increase total and LDL cholesterol was developed. Conclusions from the Seven Countries Study (Keys *et al*., 1981) led to the development of hypotheses implicating excessive consumption of saturated fats as a major contributor to the incidence of CHD. However, confounding factors in the Seven Countries Study have caused the conclusions to be questioned. There followed a number of epidemiological, mechanistic and intervention studies to provide evidence for this hypothesis.

It took some time before the different effects of saturated fatty acids varying in chain length were recognised. Short-chain (C4:0 to C6:0) or medium-chain (C8:0 to C10:0) fatty acids are directly absorbed into the portal circulation and hence have minimal effects on plasma cholesterol concentrations (Lichtenstein, 2006). Lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids were reported to increase both LDL cholesterol and HDL cholesterol (Hegsted *et al*., 1993; Katan *et al*., 1994; Clarke *et al*., 1997), whereas stearic acid (C18:0) reduced LDL cholesterol concentrations relative to C12:0, C14:0 and C16:0 (Mensink, 2005). It is now generally recognised that only C12:0 to C16:0 contribute to increases in LDL cholesterol. However, it is difficult to distinguish between the effects of C14:0 and C16:0 because they occur together in most saturated fats. In bovine milk fat, the sum of C12:0, C14:0 and

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C16:0 is typically 42–43% of the fat (Creamer & MacGibbon, 1996), with 23% of the fat being saturated, but not expected to contribute to increased plasma LDL cholesterol concentrations due to the chain length of the fatty acids. Sheep milk and goat milk are significantly different in fatty acid composition from bovine milk (Table 4.6), with higher concentrations of short-chain C4:0 and medium-chain C12:0 in sheep milk and higher concentrations of C10:0 in goat milk. Intake of dietary short- and medium-chain fatty acids is beneficial for individuals suffering from nutrient malabsorption syndromes such as cystic fibrosis, since these fatty acids have greater water solubility than long-chain fatty acids, and this allows them to be absorbed rapidly from the small intestine into the portal circulation.

Recent concern about the nutritional effects of *trans* fatty acids in the diet on risk of CHD have led many countries to require a reduction in consumption of dietary *trans* fatty acids. However, the available evidence indicates that the *trans* fatty acids in milk fat do not have adverse effects at normal levels of consumption (Uauy *et al*., 2009), and recommendations normally require reductions in consumption of *trans* fatty acids from industrial hydrogenated fats as the mechanism for reducing intake of this dietary component. It has been pointed out that any attempt to reduce intake of *trans* fatty acids from dairy products would have the consequence of reducing intake of beneficial dietary components including calcium (Scientific Advisory Committee on Nutrition, 2007).

The significance of the cholesterol content of dietary animal products in contributing to increased risk of CHD has been the subject of much debate. Biosynthesis of cholesterol in the human liver and in other tissues typically contributes about two-thirds of the circulating pools of cholesterol (Dietschy, 1984). However, dietary cholesterol has been shown to have only small effects on plasma concentrations in most individuals either by inhibition of synthesis of cholesterol or by the reduction of cholesterol absorption and increased metabolism to bile acids. In the case of infants, cholesterol biosynthesis is suppressed to a large extent by the consumption of breast milk with a high cholesterol content instead of low-cholesterol infant formula (Cruz *et al*., 1994). Much of the understanding of the effect of diet on cholesterol biosynthesis has come from animal studies. All animal cells manufacture cholesterol, with relative production rates varying by cell type and organ function.

For the majority of the human population, the amount of cholesterol ingested in the diet has a small effect on the concentrations of circulating cholesterol, and observational studies in humans have not demonstrated the cholesterol content of the diet as a major factor in increased risk of CHD (Kratz, 2005). For a small proportion of the population blood cholesterol levels are more strongly affected by the concentration of dietary cholesterol (Fernandez, 2006). However, dietary cholesterol increases the concentrations of both circulating LDL and HDL cholesterol in those individuals who demonstrate an increase in plasma cholesterol following ingestion of cholesterol, and the increase in the total/HDL cholesterol ratio is normally small.

Information about the effects of milk fat on CVD is mainly based on prospective cohort studies, case–control studies, and metabolic studies. Well-powered long-term dietary intervention trials have not been performed, so the evidence is limited.

The effects of milk fat consumption in raising plasma LDL concentrations have been shown in a number of metabolic studies, and this has been shown to be due to the saturated fatty acid content of the fat. This was clearly demonstrated in a randomised crossover intervention, in which 33 volunteers consumed either a diet containing milk, cheese, butter and ice-cream to provide 20% of energy as fat, or the same dairy products prepared from milk which had approximately 13.7% of the fatty acids present in the form of C14:0 and C16:0 replaced by unsaturated fatty acids (Noakes *et al*., 1996). The dairy products containing the more unsaturated fatty acid profile produced a significant 0.28 mmol/L (4.3%) fall in total plasma cholesterol ($P < 0.001$), with most of the fall in LDL cholesterol, whereas HDL cholesterol and triacylglycerols remained essentially unchanged at the end of the 3-week intervention with the modified dairy products.

Milk fat is mainly consumed in the form of milk or dairy products in which components other than triacylgycerols make important contributions to the nutritional effects of the product. The fat phase of milk contains the fat-soluble vitamins A, D and E and the aqueous phase contains vitamins B_6 and B_{12} as well as folate and minerals especially calcium. Dairy products are an important source of calcium. It has been proposed that the high calcium content of dairy products reduces the LDL-raising effects of saturated fatty acids by binding to, and increasing the excretion of, saturated fatty acids and bile acids. Studies by Bendsen *et al*. (2008) and Jacobsen *et al*. (2005) provided evidence of increased faecal fat excretion and this has been confirmed by a recent small study by Lorenzen and Astrup (2011), which investigated the effects of diets containing high or low dairy fat (saturated fatty acid) content in combinations with high or low calcium content in a randomised crossover design. A significant increase in serum LDL cholesterol was observed in response to the high-fat/low-calcium diet but this was reduced in volunteers who consumed a high-fat and high-calcium diet. This finding was accompanied by an increase in the faecal excretion of

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saturated fatty acids and bile acids in the high-calcium groups. In recent years increased attention has been paid to a number of other risk factors besides lipids that may be affected by dietary dairy products. These include blood pressure, inflammation, insulin resistance, type 2 diabetes, obesity and the metabolic syndrome. The term 'metabolic syndrome' refers to a combination of conditions that increase risk of CVD and diabetes. Definitions vary but the conditions include insulin resistance plus two of the following: high blood pressure or taking blood pressure medication; raised plasma triacylglycerols; low HDL cholesterol; body mass index (BMI) greater than 30 and/or waist/hip ratio >0.9 in men, >0.85 in women; urinary albumin excretion rate ≥20 μg/min or albumin/creatinine ratio ≥30 mg/g.

In a review of the effects of consumption of milk and dairy products on body weight, Dougkas *et al*. (2011) reported that epidemiological and intervention studies provide evidence of a small negative association between consumption of milk and dairy products and BMI and other measures of adiposity. In studies that have provided restricted energy, higher intake of milk and dairy products has caused increased weight loss and maintenance of lean tissue (Gilbert *et al*., 2011). Effects on lipolysis, lipogenesis and fatty acid absorption have been discussed as possible mechanisms for these effects. Effects of dairy products on reducing appetite in volunteers on a weight loss diet have also been reported (Gilbert *et al*., 2011). Potential benefits of some dairy products on blood pressure and HDL concentrations have been reported, and possible beneficial effects on obesity, the metabolic syndrome and diabetes have been discussed.

The FAO/WHO expert consultation in 2008 did not reach full agreement on the recommended maximum total fat intake, but concluded that it was prudent to maintain the recommendation of 30–35% calories as fat (FAO, 2010). They considered that there is convincing evidence that replacing saturated fatty acids (C12:0 to C16:0) with polyunsaturated fatty acids decreases the risk of CHD, but that there is probable evidence that replacing saturated fatty acids with refined carbohydrates has no benefit on CHD and may even increase the risk of CHD. They considered that increased intake of saturated fatty acids may increase the risk of diabetes. They did not feel that there was sufficient evidence to come to a conclusion on the effect of replacing saturated fatty acids with monounsaturated fatty acids or wholegrain carbohydrates, although indirect evidence suggested a reduced risk of CHD. However, an expert panel concluded that there was no clear benefit of replacing saturated fatty acids by carbohydrates and commented that few studies have addressed the question of the effect of the quality of carbohydrates (Astrup *et al*., 2011). Saturated fatty acids raise total and LDL cholesterol but recent evidence indicates that the ratio of total cholesterol to HDL cholesterol is a better predictor of risk of CHD than LDL cholesterol (Lewington *et al*., 2007).

The USDA Dietary Guidelines Advisory Committee issued *Dietary Guidelines for Americans* in 2010, which recommended reducing intake of cholesterol to less than 300 mg/day. Nutrition advice in the UK recommends no increase in dietary cholesterol without setting an upper limit. The *Dietary Guidelines for Americans* and FAO/WHO recommended reducing saturated fat to less than 10% of calories. However, the different effects of saturated fatty acids of different chain length have not been allowed for in the dietary guidelines.

4.10 EVIDENCE FOR EFFECTS OF MILK FAT ON CVD FROM PROSPECTIVE COHORT STUDIES

A meta-analysis increases statistical power by combining the results of several studies that test a set of related research hypotheses. A recent dose–response meta-analysis evaluated the findings of 17 prospective cohort studies investigating the effects of milk and dairy consumption on the incidence of CVD and all-cause mortality (Soedamah-Muthu *et al*., 2011). The prospective studies included 2283 CVD, 4391 CHD, 15 554 stroke and 23 949 mortality cases. The authors found a modest inverse association between milk intake and risk of overall CVD, with a relative risk of 0.94 with 200 mL/day intake of milk. There was no association between milk intake and risk of CHD, stroke or total mortality. There was also no association between intake of dairy products, either high fat or low fat, and CHD, although the numbers of volunteers in studies that distinguished between these two groups of consumers were limited.

Another meta-analysis (Elwood *et al*., 2010) concluded that increased dairy consumption caused a reduction in risk of mortality relative to those with the lowest intake, with the relative risk being 0.87 for all-cause deaths, 0.92 for ischaemic heart disease (IHD), 0.79 for stroke and 0.85 for incident diabetes.

The results of the Netherlands Cohort Study, a large prospective cohort study aimed at investigating the association between dairy product consumption and the risk of death (from all causes, IHD, and stroke), have been recently reported (Goldbohm *et al*., 2011). The study investigated the association between dairy product consumption and mortality in 120 000 men and women over a 10-year period during which over 16 000 people with complete dietary information had died. Multivariate survival analyses demonstrated a slightly increased risk of all-cause and IHD mortality for both butter and dairy fat intake [per 10 g/day;

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rate ratio (mortality) 1.04] only in women. Fermented full-fat milk was inversely associated with all-cause mortality in both sexes.

The Atherosclerosis Risk in Communities (ARIC) study was a prospective cohort study investigating changes in blood pressure in a population of 6912 white and 1296 African-American non-hypertensive men and women, aged 45–64 at baseline. After 9 years of follow-up, systolic blood pressure of whites consuming three or more portions of low-fat milk per day increased by 2.7 mmHg less than in those consuming less than one portion per week, but there was no association of consumption of dairy products with changes in blood pressure in African-Americans (Alonso *et al*., 2009a).

4.11 EVIDENCE ABOUT THE EFFECTS OF DAIRY PRODUCTS ON NON-LIPID RISK FACTORS

Conclusive evidence from large-scale, well-powered dietary intervention studies is lacking, but there are some studies that indicate possible beneficial effects of dairy products on blood pressure and the metabolic syndrome.

The multicentre Dietary Approach to Stop Hypertension (DASH) study was a key dietary intervention study which provided evidence that a diet rich in fruits and vegetables and low-fat dairy products reduced blood pressure (Appel *et al*., 1997). A mean reduction of 5 mmHg in systolic blood pressure and 3 mmHg in diastolic pressure on the test diet compared with control was reported. The authors claimed that about 50% of the reduction in blood pressure was due to the low-fat dairy products consumed. It has been calculated that a reduction in blood pressure of the magnitude observed in the DASH study would reduce CHD by about 15% and stroke by about 27% (Craddick *et al*., 2003).

Wang *et al*. (2008) reported that a prospective cohort study involving 28 886 women demonstrated that the intake of low-fat dairy products was inversely associated with risk of hypertension in middle-aged and older women. The same effect was not found with full-fat dairy products.

Alonso *et al*. (2009b) compared the effects of consumption of low-fat and whole-fat dairy products on blood pressure and weight in a randomised crossover trial with 45 young normotensive adults. The study involved supplementing the normal diet with 3.5 servings daily of whole-fat or low-fat dairy products during two 8-week periods, with a 4-week washout period between the interventions. The low-fat dairy group reduced their daily energy intake, but the high-fat dairy group increased their energy intake by 1264 kJ/day during the intervention, and this caused a significant increase in weight compared with the low-fat dairy group. There were no significant differences in the effects of consumption of low-fat or whole-fat dairy products on blood pressure.

Cross-sectional analyses of data from a prospective study involving 3435 men and women demonstrated that increased consumption of dairy products was associated with a lower prevalence of the metabolic syndrome, with lower diastolic blood pressure and lower increase in BMI (Fumeron *et al*., 2011). Another study with 2267 French adults reported an inverse association between weight or waist circumference and the consumption of dairy products by overweight men (Vergnaud *et al*., 2008).

4.12 CONCLUSION

Milk fat contains mainly triacylglycerols, but small concentrations of minor components are also present. Although the C12:0 to C16:0 fatty acids are considered to contribute to the elevation of LDL and total cholesterol concentrations, the shorter-chain fatty acids do not contribute to an atherogenic lipid profile. The vitamins and the CLA provide beneficial nutritional effects in humans, and dairy products include other beneficial dietary components including calcium in the aqueous phase.

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5
Milk Major and Minor Proteins, Polymorphisms and Non-protein Nitrogen*

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5.1 MILK PROTEINS

Proteins are an extremely important class of naturally occurring compounds that are essential to all life processes. They perform a variety of functions in living organisms ranging from providing structure to reproduction. Milk proteins represent one of the greatest contributions of milk to human nutrition. Proteins are polymers of 20 different amino acids that occur regularly in milk. They have a general structure, but are functionally different from each other. The content and sequence of amino acids in a protein therefore affects its properties (Fox & McSweeney, 1998).

The nitrogen-containing portions of milk can be divided into three broad fractions: casein, whey protein and non-protein nitrogen (NPN) (Rowland, 1938). Of the total milk N across a limited number of breeds, 78.50% is ssociated with the casein fraction, 16.5% with the whey protein fraction and 5.0% with the NPN fraction (Rowland, 1938).

True protein comprises proteins synthesised within the mammary gland (casein proteins), and proteins derived preformed from the blood (bovine serum albumin or BSA). In general, many researchers do not distinguish between crude protein and total protein of milk when referring to the protein content of milk. Crude protein is a better measure of total N in milk because it accounts for the NPN fraction, comprising approximately 5% of the total milk N. True protein does not account for the NPN fraction. This could be an advantage in animal breeding programmes and in the evaluation of the manufacturing properties and nutritional qualities of the milk (DePeters *et al*., 1993).

The average protein content in bovine milk has not changed much in the last 50 years, and is around 3.4–3.5%. The real protein content of milk (the difference between total protein and NPN) is, on average, 3.3%. Milk protein consists of different fractions, of which casein comprises 80% and whey or serum protein the remaining 20%. The NPN compounds comprise 5% of the total nitrogen content of milk (Alston-Mills, 1995).

The caseins can be subdivided into α-, β-, γ- and κ-caseins. The sequences of β- and γ-caseins are similar, as γ-casein originates from β-casein. The α-caseins can be grouped into α_{s0}-, α_{s1}- and α_{s2}-casein (Csapó & Csapóné, 2002). The approximate proportions of κ-, α_{s2}-, α_{s1}- and β-casein in cow milk are 1 : 1 : 4 : 4 (Wang *et al*., 2009).

Whey proteins include α-lactalbumin and β-lactoglobulin along with BSA and immunoglobulin (Wang *et al*., 2009). At present, methods to analyse the fractions of milk proteins include liquid chromatography (Bordin *et al*., 2001; Enne *et al*., 2005; Thoma *et al*., 2006), gel electrophoresis

* In honour of the memory of Prof. Dr Sándor Szakály and Dr Béla Schäffer (Hungarian Dairy Research Institute Ltd, Mosonmagyaróvár, Hungary).

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Table 5.1. Protein content of bovine milk.

%	g/L	Sources
2.9–3.1		Czerniawska-Piatkowska *et al*. (2004)
3.4–3.7		Çardak (2005)
3.4		Jílek *et al*. (2006)
3.34		Wedholm *et al*. (2006)
3.3		Zhai *et al*. (2007)
	32.0–33.2	Bobe *et al*. (2007)
3.4–3.6		Samoré *et al*. (2007)
6.3–6.8		Mech *et al*. (2008) (Mithun cows, India)
	30.7–31.5	Botaro *et al*. (2008)
3.4		Konjačić *et al*. (2010)
3.3–3.6		Król *et al*. (2010)
3.6	38.8	Bonfatti *et al*. (2010)
3.2–3.4		Bendelja *et al*. (2011)
3.5–3.7		Jonkus & Paura (2011)
	40.3	Cozma *et al*. (2011)
3.4–3.5		Pytlewski *et al*. (2011)

(Rehder-Silinski & McGown, 2003), isoelectric focusing and reversed-phase HPLC (Wang *et al*., 2009).

5.1.1 Factors affecting the protein content of the milk

The protein content in bovine milk is affected by several factors (Table 5.1): the cow, breed, feed, feeding regimes, milking technology, season, oestrus, part of the world, parity, stage of lactation, time of day, etc.

Although many breeds of cattle are milked worldwide, the most popular milk-producing dairy cattle breed is the Holstein-Friesian, and most publications have reported about its milk production traits. This breed has become the dominant milk producer in the last 30 years. In most cases, breed differences are found in the protein content of bovine milk. Studying the milk production of various Holstein crossbred cows, Czerniawska-Piatkowska *et al*. (2004) as well as Pytlewski *et al*. (2011) observed that the increase of Holstein-Friesian blood decreased the protein content of the milk. Çardak (2005) compared the milk protein content of Simmental and Holstein-Friesian cows and found significant differences between them in favour of the Simmental. A similar tendency was found by Bendelja *et al*. (2011) between Holstein and Simmental cows. Barłowska (2007) compared seven and Król *et al*. (2010) studied six Polish cattle breeds including Holstein-Friesian, and found significant breed difference in terms of milk protein content. Taken together, the milk from local cow breeds, in comparison with Holstein-Friesian cows raised under intensive conditions, is a more valuable source of functional whey proteins, especially in the summer season when animals graze outdoors. Studying the milk protein content of Mithun (*Bos frontalis*) cows in India, Mech *et al*. (2008) found much higher values. The increase in milk production via selection will also reduce the protein content in milk (Bobe *et al*., 2007). Conversely, Wedholm *et al*. (2006) did not find any significant breed deviations between Red and Black and White Holsteins in Sweden. Comparing the protein content of milk from Girolando and Holstein cows, Botaro *et al*. (2008) did not observe any significant differences.

Parity has an increasing effect on milk protein content (Bendelja *et al*., 2011; Jonkus & Paura, 2011) and the daily protein production (Wood *et al*., 2003) also increases with age up to the fifth lactation.

The season of production can also modify the protein content of the milk (Barłowska, 2007; Król *et al*., 2010; Bendelja *et al*., 2011), with higher levels in autumn and winter than in spring and summer. The diet (which could also be connected to the season) also has a significant effect on milk protein content (Zhai *et al*., 2007).

According to Ullah (2011) and Pytlewski *et al*. (2011), the stage of lactation also has an effect on protein content such that it increases with the number of lactation days. The part of the day also modifies the protein level, with lower values in the morning than in the evening (Bendelja *et al*., 2011).

The milk protein genotypes also have effects on the protein content, especially variants of β-lactoglobulin and κ-casein (Bobe *et al*., 1999; Kučerová *et al*., 2006) and those of α_{s1}-casein (Bobe *et al*., 2007) and β-casein (Çardak, 2005).

5.2 THE MAJOR MILK PROTEINS

Milk contains hundreds of types of protein, most of them in very small amounts. The proteins can be classified in various ways according to their chemical or physical properties and their biological functions. The old way of grouping milk proteins was into casein, albumin and globulin. The modern system for grouping is into caseins, whey (serum) proteins, and minor proteins including fat globule membrane proteins. These main groups of proteins in milk are distinguished by their widely different behaviours and structures. The caseins are easily precipitated from milk in a variety of ways, while serum proteins usually remain in solution (Anon., 2003).

Bovine milk has two groups of major proteins: caseins have four varieties (α, β, γ, κ), while whey proteins have two dominant and numerous small variants (Tables 5.2 and 5.3). A Chinese study examining domestic and foreign milk samples found that the average percentage concentrations of κ-casein, α_{s2}-casein, α_{s1}-casein and β-casein were 8%, 11%, 27% and 35%, respectively, with

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Table 5.2. Concentration of casein variants in cow milk.*

Casein	Concentration in milk (g/L)	Per cent of total casein	Reference
α_{s1}-Casein		30.9–32.8	Bobe *et al*. (1999)
	10.0	30.6	Anon. (2003)
		31.1–31.5	Bobe *et al*. (2007)
		26.9	Wang *et al*. (2009)
	11.38	35.61	Bonfatti *et al*. (2010)
α_{s2}-Casein		7.0–7.8	Bobe *et al*. (1999)
	2.6	8.0	Anon. (2003)
		7.6–8.0	Bobe *et al*. (2007)
		11.2	Wang *et al*. (2009)
	4.2	12.1	Bonfatti *et al*. (2010)
β-Casein	9.3	28.4	Walstra & Jenness (1984)
		27.8–29.3	Bobe *et al*. (1999)
	10.1	30.8	Anon. (2003)
		28.0–28.2	Bobe *et al*. (2007)
		35.6	Wang *et al*. (2009)
	13.0	37.1	Bonfatti *et al*. (2010)
γ-Casein	1.6	4.7	Bonfatti *et al*. (2010)
κ-Casein	3.3	10.1	Walstra & Jenness (1984)
		16.6–19.6	Bobe *et al*. (1999)
		18.4–18.7	Bobe *et al*. (2007)
		8.5	Wang *et al*. (2009)
	3.7	10.5	Bonfatti *et al*. (2010)
Total casein		80.3	Csapó & Csapóné (2002)
	26.0	79.5	Anon. (2003)
		85.7–86.0	Bobe *et al*. (2007)
	29.4	79.5	Csapó & Csapóné (2009)
	33.9		Bonfatti *et al*. (2010)
	28.3		Cozma *et al*. (2011)

*Data affected by breed, genotype, selection, season, feeding regimes, geography.

a relative proportion of 1.0 : 1.3 : 3.2 : 4.2 (Wang *et al*., 2009), and the total casein ratio varied between 80 and 83%, in agreement with the results of Bordin *et al*. (2001). The percentages of whey proteins in milk varied between 17.1 and 20.0% (Wang *et al*., 2009).

As the quantity of milk produced per cow and the percentage of protein in milk are equally important, many studies have been carried out to determine the accuracy of assays and to estimate the possible results of breeding programmes aiming to increase the production of milk and the milk proteins, especially that of the caseins.

A small to moderate heritability of milk protein composition was observed in earlier studies (Renner & Kosmack, 1975; Kroeker *et al*., 1985; Bobe *et al*., 1999). Ikonen *et al*. (2004) found very similar heritability for protein (0.29) and for casein (0.30), while in a study by Samoré *et al*. (2007) no difference was observed between them, both showing values of 0.31. The genetic and phenotypic correlations between protein and casein content (0.91–0.99 and 0.92–0.97) are very high (Ikonen *et al*., 2004; Samoré *et al*., 2007).

According to Bonfatti *et al*. (2011), the heritability of the contents of milk proteins ranges from 0.11 (α-lactalbumin) to 0.52 (κ-casein). Heritabilities for α_{s1}-casein %, κ-casein % and β-casein % were similar and ranged from 0.63 to 0.69, whereas heritability of α_{s2}-casein %, γ-casein % and β-lactoglobulin % were 0.28, 0.18 and 0.34, respectively. These data indicate that selection aiming to increase the milk yield might change the milk protein composition as well.

5.2.1 Caseins

Normal bovine milk contains about 3.5% protein, of which casein constitutes about 80%. Casein is a group name for the dominant class of proteins in milk. The

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Table 5.3. Concentration of main whey proteins in cow milk.

Whey proteins	Concentration in milk (g/L)	Per cent of total protein	Reference
α-Lactalbumin	1.2	3.7	Walstra & Jenness (1984)
		3.6–3.9	Bobe *et al*. (1999)
	1.2	3.7	Anon. (2003)
		3.7–3.8	Bobe *et al*. (2007)
	1.0–1.2		Król *et al*. (2010)
	1.2		Bonfatti *et al*. (2010)
β-Lactoglobulin	3.2	9.8	Walstra & Jenness (1984)
		9.6–12.4	Bobe *et al*. (1999)
	3.2	9.8	Anon. (2003)
	3.0–3.7		Reklewska *et al*. (2003)
	3.5		Lindmark-Månsson *et al*. (2003)
	5.5		Wedholm *et al*. (2006)
		10.4–10.5	Bobe *et al*. (2007)
	2.9–3.6		Król *et al*. (2010)
	3.7		Bonfatti *et al*. (2010)
Total whey proteins	6.3	19.3	Walstra & Jenness (1984)
		14.2–14.3	Bobe *et al*. (2007)
		17.6	Wang *et al*. (2009)
	6.4	20.5	Csapó & Csapóné (2009)
	4.9		Bonfatti *et al*. (2010)
		11.8	Cozma *et al*. (2011)

caseins easily form polymers, composed of hundreds and thousands of individual molecules that form a colloidal solution. These molecular complexes are known as casein micelles (Anon., 2003).

The casein content of milk, similarly to proteins, depends on a number of factors. The average value for so-called European breeds (*Bos taurus*) is around 2.6–2.8% in whole bovine milk (Samoré *et al*., 2007), and might vary between 19.76 and 40.79 g/L (Bonfatti *et al*., 2010; Cozma *et al*., 2011). Comparing the milk traits of Girolando and Holstein-Friesian cows (2.07–2.12 vs. 2.095–2.13 g/100 g), Botaro *et al*. (2008) did not find significant differences. They also observed seasonal deviations, with lower levels in rainy seasons (1.91–2.06 g/100 g) and higher levels in dry seasons (2.11–2.15 g/100 g), independent of breed; however, the effect of β-lactoglobulin variants was different.

The farm effect and the ratio of Holstein-Friesian blood (75–100%) could also modify (2.31–2.47%) the protein percentage (Czerniawska-Piatkowska *et al*., 2004). In studies of seven (Reklewska *et al*., 2003) and six (Król *et al*., 2010) dairy cattle breeds (including local and Holstein-Friesian breeds) in Poland, the casein percentage in whole milk (2.30–22.64%) showed a strong breed and seasonal effect, while Wedholm *et al*. (2006) did not find much of a breed effect on the casein ratio in the milk of Swedish and Danish Holstein cows. Several studies on urea and casein contents have indicated the existence of variability among cows (Wood *et al*., 2003; Ikonen *et al*., 2004; Mitchell *et al*., 2005). The average concentration in total protein is about 80%, but this value can be modified by selection (85.74–86.01%; Bobe *et al*., 2007). Breeding work aiming to increase milk yield reduces the concentration and the ratio of the protein, but the proportions of α_{s1}-casein (0.29–0.39%) could be increased. Other breeds may have much higher casein content, for example casein content can reach 4.04–4.77% in the milk of Indian Mithun cows (*Bos frontalis*) (Mech *et al*., 2008; Meehl *et al*., 2010).

Casein, as a proportion of total milk proteins, is higher during early lactation stages, while the proportion of α_s-, β- and κ-casein in total casein decreases systematically during lactation (Alomirah *et al*., 2000; Jílek *et al*., 2006; Ullah, 2011), and the time of day also affects the value (Meehl *et al*., 2010).

The caseins in milk of the genus *Bos* were defined originally by Jenness *et al*. (1956) as those phosphoproteins that precipitate from raw skimmed milk by acidification to pH 4.6 at 20 °C. In a subsequent report (Whitney *et al*.,

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1976), the caseins were differentiated (by a committee) according to their relative electrophoretic mobility in alkaline polyacrylamide or starch gels containing urea with or without mercaptoethanol (Farrell *et al*., 2004; reviewed by Caroli *et al*., 2009).

5.2.1.1 α_{s1}-Casein

The α_{s1}-casein family, which constitutes up to 40% of the casein fraction in bovine milk, consists of one major and one minor component (Farrell *et al*., 2004). Both proteins are single-chain polypeptides with the same amino acid sequence (Mercier *et al*., 1971; Grosclaude *et al*., 1973) and differ only in their degree of phosphorylation. The minor component contains one additional phosphorylated serine residue at position 41 (Eigel *et al*., 1984). The reference protein for this family is α_{s1}-casein B-8P, a single-chain protein with no cysteinyl residues. The F variant in German Black and White cattle (Erhardt 1993), the G variant in Italian Brown cows (Mariani *et al*., 1995), and the H variant (Mahé *et al*., 1999) have also been identified.

The variant A is found in Holstein-Friesians, Red Holsteins and German Red cattle (Ng-Kwai-Hang *et al*., 1984; Grosclaude, 1988; Erhardt, 1993), while the B variant is predominant in *Bos taurus* (Eigel *et al*., 1984); the C variant is found in *Bos indicus* and *Bos grunniens* (Eigel *et al*., 1984); the D variant occurs in various breeds in France (Grosclaude, 1988), Italy (Mariani & Russo, 1975) and the Netherlands (Jersey; Corradini, 1969); and the E variant is found in *Bos grunniens* (Grosclaude *et al*., 1976). Cows carrying the G allele produce less (in the case of GG genotype, 55%) α_{s1}-casein and more of the other caseins (Mariani *et al*., 1995). The α_{s1}-casein BB phenotype has been correlated with higher milk yields and higher protein yields over the course of lactation (Ng-Kwai-Hang *et al*., 1984; Aleandri *et al*., 1990; Sang *et al*., 1994), but the same phenotype has also been correlated with lower protein concentrations in milk (Ng-Kwai-Hang *et al*., 1986; Ng-Kwai-Hang & Grosclaude, 1992).

5.2.1.2 α_{s2}-Casein

The α_{s2}-casein family contributes up to 10% of the casein fraction in bovine milk (Caroli *et al*., 2009), and consists of two major and several minor components exhibiting varying levels of post-translational phosphorylation (Swaisgood, 1992) and minor degrees of intermolecular disulphide bonding (Rasmussen *et al*., 1992). The predominant forms in bovine milk contain an intramolecular disulphide bond and differ only in their degree of phosphorylation. The α_{s2}-casein appears to be readily susceptible to proteolysis as assessed by the activities of chymosin and plasmin towards the protein.

5.2.1.3 β-Casein

The β-casein family constitutes up to 45% of the casein of bovine milk (Farrell *et al*., 2004) and is quite complex because of the action of the native milk protease plasmin (Eigel *et al*., 1984). Plasmin cleavage leads to formation of γ_1-, γ_2-. and γ_3-casein, which are actually fragments of β-casein.

5.2.1.4 κ-Casein

The κ-casein family consists of a major carbohydrate-free component and a minimum of six minor components (Farrell *et al*., 2004). The six minor components, as detected by polyacrylamide gel electrophoresis in urea with 2-mercaptoethanol (Mackinlay & Wake, 1965; Pujolle *et al*., 1966; Woychik *et al*., 1966; Vreeman *et al*., 1977; Doi *et al*., 1979), represent varying degrees of phosphorylation and glycosylation. The κ-casein isolated from milk also occurs in the form of a mixture of disulphide-bonded polymers ranging from dimers to octamers and above (Groves *et al*., 1992).

The κ-casein constitutes approximately 12% of the total casein, and due to its role in the regulation of micelle size and milk properties, it has been characterised across different species of the *Bos* genus, especially in different cattle breeds. Many studies on the κ-casein gene have indicated that certain milk protein variants may be associated with milk production, milk composition and cheese production (Comin *et al*., 2008; Caroli *et al*., 2009). The B variant is associated with thermal resistance, shorter coagulation time, better curdles and improved micelle size, while the A variant is associated with higher milk yield. The milk from cows with the BB genotype gives a 10% higher yield of cheese compared with the milk from cows with the AA genotype (Azevedo *et al*., 2008).

5.2.1.5 The question of casein structure

The largest structures in the fluid portion of milk are casein protein micelles, which are aggregates of several thousand protein molecules bonded with the help of nanometre-scale particles of calcium phosphate. Each micelle is roughly spherical and about 0.1 μm across. There are several competing hypotheses regarding the precise structure of the micelles, but they share one important feature: the outermost layer consists of strands of one type of protein, κ-casein, reaching out from the body of the micelle into the surrounding fluid. These κ-casein molecules all have a negative electrical charge and therefore repel each other, keeping the micelles separated under normal conditions and in stable colloidal suspension in the water-based surrounding fluid (Alston-Mills, 1995; Fox & McSweeney, 1998; McGee, 2004).

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For many years the most accepted hypopthesis regarding the structure of a micelle implicated 'submicelles' (composed of spherical casein aggregates) that were held together by calcium phosphate linkages. The Walstra–Jennes model (Walstra & Jenness, 1984) is based on the inclusion of many known facts about casein micelle behaviour and is presented in most books and articles. However, there is no universal acceptance of this model among dairy scientists. In fact there is mounting evidence that well-defined casein submicelles do not exist; rather the structure is more open and fluid, perhaps a 'bowl-of-spaghetti' type model (Walstra, 1999). The big problem with the earlier model was the distribution of calcium phosphate, and it is certainly apparent now that calcium phosphate is more evenly distributed throughout the micelle so, based on the Walstra–Jennes model, is found both within and outside the submicelles (McGee, 2004).

One of the hypotheses, attributed to DeKruif and Holt (2003), proposes that nanoclusters of calcium phosphate and the phosphopeptide fraction of β-casein are the centrepiece of the micellar structure. Specifically in this view, unstructured proteins organise around the calcium phosphate, giving rise to their structure and thus no specific structure is formed (DeKruif & Holt, 2003).

The other model suggests a more or less spherical, highly hydrated, and fairly open particle (Holt & Horne, 1996). Polypeptide chains in the core are partly cross-linked by nanometre-sized clusters of calcium phosphate; the internal structure gives rise to an external region of lower segment density known as the hairy layer, which confers steric and/or charge stability to native casein particles.

The previous hypothesis was partly further developed and proposed by Horne (Horne, 1998, 2002), and stated that the growth of calcium phosphate nanoclusters begins the process of micelle formation, but is limited by binding the phosphopeptide loop regions of the caseins. Once bound, protein–protein interactions are formed and polymerisation occurs, in which κ-casein is used as an end cap, to form micelles with trapped calcium phosphate nanoclusters.

Using stereo images generated by an electron microscope, a model of casein supramolecular structure was developed (McMahon & Oommen, 2007). The model satisfies the principles observed in nature, such as self-aggregation, interdependence and diversity. The model indicates how the casein supramolecule is an irregular structure that allows for all possible combinations of proteins. The calcium phosphate is formed into clusters, because of low solubility, and is prevented from nucleating into crystals by being rapidly bound by the calcium-sensitive caseins. These calcium phosphate nanoclusters act as nodes that hold the protein chains together.

The casein aggregate, comparable to a sphere of approximately 100 nm in diameter, has been studied over the past 30 years using physicochemical methods. However, its structure and thus its molecular and supramolecular organisation are still poorly understood. This lack of knowledge is a major conceptual obstacle for understanding and therefore controlling its functionalities. In fact, the casein micelle plays a key role in many transformation processes in the food industry. The various kinds of casein structural models were recently reviewed by Dalgleish (2011).

5.2.1.6 The importance of casein structure

The caseins, being divided into the four groups α_{s1}-, α_{s2}-, β- and κ-caseins, are very heterogeneous and consist of a good number of genetic variants (see Table 5.4). These variants differ from each other only by a few amino acids (Farrell *et al*., 2004; Caroli *et al*., 2009). The amino acids of α- and β-casein are esterified to phosphoric acid and bind calcium (which is abundant in milk) to form bonds between and within molecules, so caseins easily form polymers containing several identical or different types of caseins. Because of the abundant phosphate groups and hydrophobic sites in the casein molecule, the molecular polymers formed by the caseins are very special and stable. The polymers are composed of hundreds and thousands of individual molecules and form a colloidal solution, which is what gives milk its white colour. These molecular complexes are known as casein micelles (Dalgleish, 2011).

The casein complexes (micelles) are extremely important in milk manufacturing processes. The calcium phosphate and hydrophobic interactions between submicelles are responsible for the stability of casein micelles. The hydrophilic parts of κ-casein contain carbohydrate groups, which project from the outside of the complex micelles, giving them a 'hairy' appearance, but more importantly they stabilise the micelles against aggregation. Rennet, used in the first stage of the cheesemaking process, splits the carbohydrate of the κ-casein on the surfaces of the micelles. Therefore, the micelles lose their solubility and start to aggregate to form a curd. At low temperature the structure of the micelle is weakened, as κ-casein chains start to dissociate and the calcium hydroxyphosphate leaves the micelle structure, because β-casein is the most hydrophobic casein and the hydrophobic interactions are weakened when the temperature is lowered. Hydrolysis of β-casein to γ-casein and proteose peptones (breakdown products) means lower yield during cheese production because the proteose-peptone fractions are lost in the whey (Anon., 2005).

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5.2.2 Whey (serum) proteins

Whey protein comprises the group of proteins in whey during the cheesemaking process. Whey protein also contains fragments of casein molecules. Some milk serum proteins are also present in whey in lower concentrations than in the original milk. This is due to heat denaturation during pasteurisation of the milk prior to cheesemaking (Anon., 2003). Whey proteins in general, and α-lactalbumin in particular, have very high nutritional values. Their amino acid composition is very close to that which is regarded as a biological optimum. Whey protein derivatives are widely used in the food industry (Anon., 2003).

The main serum protein fractions are α-lactalbumin, β-lactoglobulin, serum albumin, globulins, and miscellaneous proteins and polypeptides, with the first two comprising 80% of serum protein (Csapó & Csapóné, 2002, 2009). According to various studies the percentage of whey protein (0.57–0.66%) in milk is affected by the farm and the genotype of the cow (Czerniawska-Piatkowska *et al*., 2004), the selection (Bobe *et al*., 2007), the breed and the season (Csapó & Csapóné, 2002, 2009; Król *et al*., 2010). The whey concentration in milk can vary between 4.91 and 6.4 g/L, while its ratio within total milk protein can vary between 11.79 and 20.5% (Table 5.3).

Selection for higher milk production can increase the yield and reduce the concentration of the protein, as well as the ratio of the milk proteins (Bobe *et al*., 2007). However, the increased milk yield has significant positive effects only on the proportions of α_{s1}-casein (+0.29–0.39%), while it has smaller effects on the values of other milk proteins, including caseins and whey proteins.

5.2.2.1 α-Lactalbumin

This protein may be considered to be the typical whey protein. It is present in milk from all mammals and plays a significant part in the synthesis of lactose in the udder (Anon., 2003). The second most prevalent protein in whey is α-lactalbumin, which comprises about 2% of the total milk protein and about 13% of the total whey protein. It has also been identified as a calcium metalloprotein (Hiraoka *et al*., 1980).

In dairy cattle, the concentration of α-lactalbumin in milk decreases near the end of lactation (Wu & Satter, 2000; Jílek *et al*., 2006; Ullah, 2011). This is in contrast to what occurs with the other major bovine milk proteins; their concentrations tend to increase as lactation progresses (Davies & Law, 1980). The decline in α-lactalbumin concentration is correlated with the decline observed in the concentration of milk lactose at the end of lactation. Lower concentrations of α-lactalbumin have also been observed in cows that have mammary infections (Caffin *et al*., 1985).

Its concentration in milk varies between 0.98 and 1.25 g/L and it comprises 3.6–3.9% of the total protein (Table 5.3). This value is affected by breed, season (Lindmark-Månsson *et al*., 2003; Reklewska *et al*., 2003; Wedholm *et al*., 2006; Król *et al*., 2010) and selection (Bobe *et al*., 1999, 2007).

5.2.2.2 β-Lactoglobulin

This protein is found only in ungulates and is the major whey protein component of cow milk (Anon., 2003). β-Lactoglobulin comprises 10% of total milk protein or about 58% of whey protein. Currently there is no clear function identified for β-lactoglobulin, although it does act as a transporter of fatty acids and retinol and may play a role in enzyme regulation and passive immunity of neonates (Hambling *et al*., 1992; Pérez & Calvo, 1995; Kontopidis *et al*., 2004), but primarily it is an important source of amino acids.

Its concentration in milk can vary within a wide range (2.93–5.5 g/L), while it comprises 9.56–12.4% of total milk protein according to various authors (Table 5.3). The values are strongly modified by several factors, such as breed (Reklewska *et al*., 2003; Wedholm *et al*., 2006; Król *et al*., 2010), season and available feed (Król *et al*., 2010), and selection (Bobe *et al*., 1999). The quantity declines at the end of the lactation (Wu & Satter, 2000; Jílek *et al*., 2006; Ullah, 2011).

5.3 THE POLYMORPHISMS OF MILK PROTEINS

Studies on milk protein genetic variability started more than 50 years ago with the detection of the main β-lactoglobulin variants (Aschaffenburg & Drewry, 1957) and intensified during the 1990s with the discovery of polymorphisms with important differences among bovine species and breeds (Formaggioni *et al*., 1999). This polymorphism in milk proteins has raised great interest in animal breeding and the dairy industry, because of the relationship between milk proteins and milk production traits, composition and quality (Caroli *et al*., 2004; DeMarchi *et al*., 2008).

The three caseins (α_{s1}, β and κ) and β-lactoglobulin of bovine milk exhibit genetic polymorphisms (very low in the case of α-lactalbumin) as a consequence of either substitution or deletion of amino acids within the polypeptide chain (Table 5.4). Because these polymorphic forms, sometimes known as genetic variants, are easily revealed by electrophoretic techniques and the corresponding genes are simply inherited according to the Mendelian mode of inheritance, milk protein genes can be used as markers for economically important traits (Eigel *et al*., 1984). Several reports have indicated that certain milk protein variants may be

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Table 5.4. Genetic variants of the main milk proteins.

Protein fractions	Variants	Reference
α-Casein	A, B, C, D	Csapó & Csapóné (2002)
α_{s1}-Casein	A, B, C, D, E, F, G, H	Baranyi *et al*. (1993); Farrell *et al*. (2004)
α_{s1}-Casein	A, B, C, D, E, F, G, H, I	Caroli *et al*. (2009)
α_{s2}-Casein	A, B, C, D	Farrell *et al*. (2004); Caroli *et al*. (2009)
β-Casein	A^1, A^2, A^3, B, Bz, ($B1_2$), C, D, E	Baranyi *et al*. (1993); Csapó & Csapóné (2002)
β-Casein	A^1, A^2, A^3, B, C, D, E, F, G, H^1, H^2, I	Farrell *et al*. (2004); Caroli *et al*. (2009)
κ-Casein	A, B	Csapó & Csapóné (2002)
κ-Casein	A, B, C, E	Baranyi *et al*. (1993)
κ-Casein	A, B, C, E, F^1, F^2, G^1, G^2, H, I, J	Farrell *et al*. (2004)
κ-Casein	A, A^1, B, B^2, C, D, E, F^1, F^2, G^1, G^2, H, I, J	Caroli *et al*. (2009)
γ-Casein	A, B	Groves & Kiddy (1968)
γ-Casein	A^1, A^2, A^3, B	Csapó & Csapóné (2002)
β-Lactoglobulin	A, B, C, D	Csapó & Csapóné (2002)
β-Lactoglobulin	A, B, C, D, E, F, G, H, W	Baranyi *et al*. (1993)
β-Lactoglobulin	A, B, C, D, E, F, G, H, I, J, W	Farrell *et al*. (2004); Caroli *et al*. (2009)
α-Lactalbumin	A, B, C	Baranyi *et al*. (1993); Csapó & Csapóné (2002); Farrell *et al*. (2004); Caroli *et al*. (2009)

associated with milk production and composition (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1984, 1986; Lin *et al*., 1986, 1989; Gonyon *et al*., 1987; Haenlein *et al*., 1987), the heat stability of milk, rennet coagulation capacity, and resistance against mastitis (Csapó & Csapóné, 2002).

Milk protein variants have been used for breed characterisation (Moazami-Goudarzi *et al*., 2001; Ceriotti *et al*., 2004), biodiversity investigations (Lien *et al*., 1999; Mahé *et al*., 1999) and evolution studies on both animal resources and milk protein genes (Jann *et al*., 2004; Ibeagha-Awemu *et al*., 2007).

Several studies have been carried out to determine the frequencies of genetic variants of milk protein in different cattle breeds (Ng-Kwai-Hang *et al*., 1990; Baranyi *et al*., 1993, 1996; Erhardt, 1996; Lien *et al*., 1999; Jeichitra *et al*., 2003; Caroli *et al*., 2004; Baranyi & Bösze, 2009; Pacheco Contreras *et al*., 2011) and possible associations between milk protein polymorphism and economically important traits like milk production, milk composition and cheese-making properties have been widely investigated (Marziali & Ng-Kwai-Hang, 1986; Pagnacco & Caroli, 1987; Aleandri *et al*., 1990; Van Eenennaam & Medrano, 1991; Caroli *et al*., 2004, 2009; Farrell *et al*., 2004) because of the potential use of milk protein types as an aid to genetic selection.

A review by Caroli *et al*. (2009) summarising genetic polymorphism of milk proteins in the *Bos* genus found 53 identified variants, of which 13 are widely distributed in *Bos taurus* and *Bos indicus*, 33 are less common or rare in these two species, and seven occur only in *Bos grunniens* and/or *Bos javanicus*. Underlining the importance of most common variants among *Bos* species and breeds, these authors stated that all can be affected by the biochemical interactions among caseins and whey proteins.

5.3.1 The presence of polymorphisms in cattle populations

In the California Holstein cattle population, the α_{s1}-casein B variant is the most common in all the breeds tested (Holstein, Milking Shorthorn, Jersey, Brown Swiss and Guernsey), while Milking Shorthorns have been found to be monomorphic for this allele. The frequency of the α_{s1}-casein C allele is highest in Jerseys (Table 5.5). The rare β-casein C allele is found exclusively in the heterozygous β-casein AC form in a small number of Guernsey cows. The β-casein A allele is the most prevalent in all the breeds (Holstein, Jersey, Brown Swiss, Guernsey and Milking Shorthorn), and a small number of samples from Holsteins carry the rare β-casein A^3 variant. All the β-casein A alleles in Guernseys are the β-casein A^2 variant. The other four breeds carry both the β-casein A^1 and the β-casein A^2 variants; the latter predominates in most of the breeds. The highest frequency of the β-casein B allele is found in Jerseys. The κ-casein A genetic variant is most frequent in Guernseys, Milking Shorthorns and Holsteins, whereas the κ-casein B allele has a higher frequency in Jerseys and Brown Swiss. The frequency of the β-lactoglobulin B allele is moderate and is the predominant allele in all five dairy breeds (Holstein, Jersey, Brown Swiss, Guernsey,

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Table 5.5. Frequency of α_{s1}-casein genetic variants.

Genotype				Allele					
AB	**BB**	**BC**	**CC**	**A**	**B**	**C**	**I**	**Breed**	**Reference**
0.23	0.97	0.026						Holstein-Friesian	Ng-Kwai-Hang *et al*. (1986, 1990)
	0.96	0.035						Holstein-Friesian	Aleandri *et al*. (1990)
					0.99	0.01		Holstein Friesian	Van Eenennaam & Medrano (1991)
					0.86	0.14		Brown Swiss	Van Eenennaam & Medrano (1991)
					0.88	0.12		Guernsey	Van Eenennaam & Medrano (1991)
					1.00			Milking Shorthorn	Van Eenennaam & Medrano (1991)
					0.68	0.32		Jersey	Van Eenennaam & Medrano (1991)
				0	0.89			Hungarian Spotted	Baranyi *et al*. (1993)
				0	0.82			Hungarian Grey	Baranyi *et al*. (1993)
					0.11	0.89		Gyr Zebu	Da Silva & Del Lama (1997)
					0.06	0.94		Guzerat Zebu	Da Silva & Del Lama (1997)
					0.14	0.86		Sindi Zebu	Da Silva & Del Lama (1997)
					0.00	1.00		Nelore Zebu	Da Silva & Del Lama (1997)
	0.0064	0.14	0.85			0.92		Kangayam cattle	Jeichitra *et al*. (2003)
	0.96	0.038			0.98	0.019		Holstein-Friesian	Çardak (2005)
	0.87	0.12	0.006		0.93	0.068		Simmental	Çardak (2005)
	0.86	0.14			0.86	0.14		Holstein-Friesian	Oner & Elmaci (2006)
	0.80	0.18	0.016		0.89	0.11		Czech Fleckvieh	Kučerová *et al*. (2006)
					0.940	0.060		Romanian Simmental	Bâlteanu *et al*. (2010)
					0.998	0.002		Romanian Black & White	Bâlteanu *et al*. (2010)
					1.00	0.000		Red Holstein	Bâlteanu *et al*. (2010)
					0.95	0.049		Brown	Bâlteanu *et al*. (2010)
					0.69	0.31		Black Pinzgau	Bâlteanu *et al*. (2010)
					0.77	0.19	0.041	Grey Steppe	Bâlteanu *et al*. (2010)
				0.010	0.97	0.020		Turkish Black & White	Gurcan (2011)

Milking Shorthorn). Only β-lactoglobulin A and B alleles are found in Jerseys (Van Eenennaam & Medrano, 1991). This differs from Australian data in which a third allele, β-lactoglobulin C, has been detected (McLean *et al*., 1984). In the Holstein, Jersey and Brown Swiss breeds, a greater than expected number of animals are heterozygous ($P<0.05$) (Van Eenennaam & Medrano, 1991).

In Dutch-Friesian and Holstein-Friesian crossbred animals the α_{s1}-casein BB and BC genotypes are observed. As in most Western dairy cattle breeds, for α_{s2}-casein only the AA genotype was found. For β-casein, the genotypes A^1A^1, A^1A^2, A^2A^2, A^1B, A^2B, BB, A^1A^3, A^2A^3 and A^3B have been found. For both κ-casein and β-lactoglobulin, the AA, AB and BB genotypes have been observed (Bovenhuis *et al*., 1992).

In Hungarian Spotted and Hungarian Grey cattle breeds the α_{s1}-casein A variant is absent, while B is dominant. Among the seven known genetic variants of β-casein, A^1 and A^2 are the most frequent in Hungarian Grey and Hungarian Spotted breeds. Dairy producers might have conceivably selected indirectly for α_{s1}-casein B and for β-casein A^1 or A^2 because selection has traditionally focused on high milk and fat yields. The examined Hungarian Spotted population showed no significant differences in the different milk protein gene frequencies compared with those of the Simmental breed, which has had the most important role in the development of the Hungarian Spotted breed. The κ-casein C variant occurs in 3% of Hungarian Spotted cows. The genotypic frequencies of κ-casein AA and AB are significantly different in the two breeds examined. The E variant of κ-casein has not been found in the Hungarian Spotted breed (Baranyi *et al*., 1993; Baranyi & Bösze, 2009) (Tables 5.6 and 5.7).

In Holstein cattle, Oner and Elmaci (2006) stated that the β-casein AA genotype is the most common, but no BB

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Table 5.6. Frequency of β-casein allele variants.

Allele							
A	**A^1**	**A^2**	**A^3**	**B**	**C**	**Breed**	**Reference**
	0.43	0.55		0.02		Holstein-Friesian	Van Eenennaam & Medrano (1991)
	0.18	0.66		0.16		Brown Swiss	Van Eenennaam & Medrano (1991)
		0.96			0.04	Guernsey	Van Eenennaam & Medrano (1991)
	0.49	0.49		0.02		Milking Shorthorn	Van Eenennaam & Medrano (1991)
	0.17	0.50		0.33		Jersey	Van Eenennaam & Medrano (1991)
	0.21	0.72		0.06	0.01	Hungarian Spotted	Baranyi *et al*. (1993)
	0.23	0.75		0.02	0	Hungarian Grey	Baranyi *et al*. (1993)
	0.01	0.94		0.052	0.002*	Gyr Zebu	Da Silva & Del Lama (1997)
	0.02	0.93		0.045	0.005*	Guzerat Zebu	Da Silva & Del Lama (1997)
	0	0.86		0.14	0*	Sindi Zebu	Da Silva & Del Lama (1997)
	0.03	0.97		0	0*	Nelore Zebu	Da Silva & Del Lama (1997)
	0.43	0.52		0.047		Holstein-Friesian	Lien *et al*. (1999)
	0.33	0.67				Icelandic	Lien *et al*. (1999)
	0.51	0.49				Norwegian	Lien *et al*. (1999)
	0.41	0.59				Swedish Black & White	Lien *et al*. (1999)
	0.40	0.60				Swedish Red & White	Lien *et al*. (1999)
	0.50	0.50				Ayshire	Lien *et al*. (1999)
	0.40	0.60				Polish Black & White	Kaminski *et al*. (2002)
0.93				0.074		Kangayam cattle	Jeichitra *et al*. (2003)
	0.11	0.69		0.18		Brown Italian	Boettcher *et al*. (2004a)
	0.38	0.55		0.07		Italian Holstein-Friesian	Boettcher *et al*. (2004a)
	0.30	0.67	0.006	0.032		Holstein-Friesian	Çardak (2005)
	0.39	0.46	0.008	0.12	0.023	Simmental	Çardak (2005)
0.966				0.034		Holstein-Friesian	Oner & Elmaci (2006)
	0.18	0.81	0.006	0.008		Czech Fleckvieh	Kučerová *et al*. (2006)
	0.34	0.60		0.06		Swedish Black & White	Hallén *et al*. (2008)
	0.44	0.55		0.01		SRBH	Hallén *et al*. (2008)
	0.36	0.63		0.01		SRBL	Hallén *et al*. (2008)
	0.34	0.57	0.003	0.044	0.047	Romanian Simmental	Bâlteanu *et al*. (2010)
	0.31	0.65		0.039		Romanian Black & White	Bâlteanu *et al*. (2010)
	0.077	0.85		0.077		Red Holstein	Bâlteanu *et al*. (2010)
	0.20	0.70		0.097	0.007	Brown	Bâlteanu *et al*. (2010)
	0.12	0.79		0.097	0.038	Black Pinzgau	Bâlteanu *et al*. (2010)
	0.48	0.40		0.12		Red Pinzgau	Bâlteanu *et al*. (2010)
	0.35	0.56		0.063	0.20	Grey Steppe	Bâlteanu *et al*. (2010)
0.95				0.050		Turkish Black & White	Gurcan (2011)

*Allele D

animals were found. Previous studies support the findings for these gene frequencies in Holstein cattle populations (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1984; Lin *et al*., 1986; Gonyon *et al*., 1987; Bonvillani *et al*., 2000) (Table 5.7).

High allele frequencies for α_{s1}-casein B, β-casein A and κ-casein A and a moderate frequency for β-lactoglobulin B were observed in the Holstein population. The α_{s1}-casein showed no AA genotype. The α_{s1}-casein B allele was found to be predominant. As for β-casein, the most common genotype is AA. The β-casein B allele has been found exclusively in heterozygous β-casein AB genotypes and there were no BB animals. The κ-casein A allele is the most common allele and the κ-casein AA genotype is more frequent than the AB and BB genotypes (Table 5.8). About half of the cows were found to be heterozygous (AB) at the β-lactoglobulin

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Table 5.7. Frequency of β-casein genotype variants.

Genotype													
A^1A^1	A^1A^2	A^1A^3	A^1B	A^1C	A^2A^2	A^2A^3	A^2B	A^2C	A^3A+	A^3B	AB	BB	Breed	Reference
0.28	0.50	0.67	0.011		0.18	0.43	0.014	0.041	0.01	0.04		0.01	Holstein-Friesian	Ng-Kwai-Hang *et al*. (1990)
											0.12	0.86	Kangayam cattle	Jeichitra *et al*. (2003)
0.059	0.46		0.013		0.41	0.008	0.046			0.004			Holstein-Friesian	Çardak (2005)
0.14	0.38	0.006	0.085	0.017	0.19	0.011	0.13	0.028				0.011	Simmental	Çardak (2005)

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Table 5.8. Frequency of κ-casein genetic variants.

Genotype					Allele					
AA	**AB**	**BB**	**EE**	**AH**	**A**	**B**	**C**	**E**	**Breed**	**Reference**
0.64	0.41	0.045							Holstein-Friesian	Aleandri *et al*. (1990)
0.54	0.42	0.039							Holstein-Friesian	Ng-Kwai-Hang *et al*. (1990)
					0.82	0.18			Holstein-Friesian	Van Eenennaam & Medrano (1991)
					0.33	0.67			Brown Swiss	Van Eenennaam & Medrano(1991)
					0.73	0.27			Guernsey	Van Eenennaam & Medrano (1991)
					0.89	0.11			Milking Shorthorn	Van Eenennaam & Medrano (1991)
					0.14	0.86			Jersey	Van Eenennaam & Medrano (1991)
0.68	0.28	0.034							Holstein-Friesian	Bobe *et al*. (1999)
0.48	0.51	0.013			0.73	0.27			Holstein-Friesian	Çardak (2005)
0.43	0.49	0.079			0.68	0.32			Simmental	Çardak (2005)
					0.71	0.29			Holstein-Friesian	Oner & Elmaci (2006)
					0.60	0.38		0.024	Czech Fleckvieh	Kučerová *et al*. (2006)
					0.95	0.055			Gyr Zebu	Da Silva & Del Lama (1997)
					0.80	0.20			Guzerat Zebu	Da Silva & Del Lama (1997)
					0.66	0.34			Sindi Zebu	Da Silva & Del Lama (1997)
					0.97	0.03			Nelore Zebu	Da Silva & Del Lama (1997)
					0.68	0.31	0.008		Romanian Simmental	Bâlteanu *et al*. (2010)
					0.84	0.16			Romanian Black & White	Bâlteanu *et al*. (2010)
					0.77	0.23			Red Holstein	Bâlteanu *et al*. (2010)
					0.37	0.62	0.011		Brown	Bâlteanu *et al*. (2010)
					0.67	0.34			Black Pinzgau	Bâlteanu *et al*. (2010)
					0.53	0.47			Red Pinzgau	Bâlteanu *et al*. (2010)
					0.42	0.58			Grey Steppe	Bâlteanu *et al*. (2010)
0.58	0.34	0.080							Romanian Simmental	Rashydov *et al*. (2010)
0.51	0.36	0.13			0.68	0.32			Turkish Black & White	Gurcan (2011)
0.14	0.51	0.36			0.40	0.60			Charollais	Pacheco Contreras *et al*. (2011)
0.11	0.60	0.29			0.41	0.59			Carora	Pacheco Contreras *et al*. (2011)
0.66	0.27		0.02	0.05	0.82	0.13	0.02	0.02	Gyrtholando	Pacheco Contreras *et al*. (2011)
0.551	0.29	0.16			0.69	0.31			Holstein-Friesian	Ren *et al*. (2011)
	0.23	0.77			0.11	0.88			Jersey	Ren *et al*. (2011)

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Table 5.9. Frequency of β-lactoglobulin genetic variants.

Genotype							Allele					
AA	**AB**	**AC**	**AD**	**BB**	**BC**	**DB**	**A**	**B**	**C**	**D**	**Breed**	**Reference**
0.11	0.49			0.40							Holstein-Friesian	Ng-Kwai-Hang *et al.* (1990)
							0.43	0.57			Holstein-Friesian	Van Eenennaam & Medrano (1991)
							0.39	0.61			Brown Swiss	Van Eenennaam & Medrano (1991)
							0.21	0.79			Guernsey	Van Eenennaam & Medrano (1991)
							0.31	0.69			Milking Shorthorn	Van Eenennaam & Medrano (1991)
							0.37	0.63			Jersey	Van Eenennaam & Medrano (1991)
							0.47	0.50		0.05	Hungarian Spotted	Baranyi *et al.* (1993)
							0.20	0.75		0.05	Hungarian Grey	Baranyi *et al.* (1993)
							0.36	0.64			Gyr Zebu	Da Silva & Del Lama (1997)
							0.14	0.86			Guzerat Zebu	Da Silva & Del Lama (1997)
							0.045	0.96			Sindi Zebu	Da Silva & Del Lama (1997)
							0.44	0.56			Nelore Zebu	Da Silva & Del Lama (1997)
0.12	0.48			0.40							Holstein-Friesian	Bobe *et al.* (1999)
0.013	0.12	0.006		0.85	0.019		0.074	0.91	0.013		Kangayam cattle	Jeichitra *et al.* (2003)
0.15	0.47			0.38			0.38	0.62			Holstein-Friesian	Çardak (2005)
0.26	0.46		0.006	0.26		0.011	0.49	0.50	0.008		Simmental	Çardak (2005)
							0.43	0.57			Holstein	Oner & Elmaci (2006)
							0.51	0.49			Czech Fleckvieh	Kučerová *et al.* (2006)
0.031	0.28			0.69			0.17	0.83			Sahiwal	Rachagani *et al.* (2006)
0.023	0.73			0.24			0.39	0.61			Tharparkar	Rachagani *et al.* (2006)
0.33	0.28			0.39			0.46	0.53			Holstein-Friesian	Botaro *et al.* (2008)
0.21	0.34			0.45			0.38	0.62			Girolando	Botaro *et al.* (2008)
0.26	0.54			0.20			0.53	0.47			Holstein-Freisian	Heidari *et al.* (2009)
							0.52	0.48	0.004		Romanian Simmental	Bâlteanu *et al.* (2010)
							0.43	0.57			Romanian Black & White	Bâlteanu *et al.* (2010)
							0.23	0.77			Red Holstein	Bâlteanu *et al.* (2010)
							0.48	0.52			Brown	Bâlteanu *et al.* (2010)
							0.27	0.73			Black Pinzgau	Bâlteanu *et al.* (2010)
							0.27	0.65	0.083		Red Pinzgau	Bâlteanu *et al.* (2010)
							0.54	0.46			Grey Steppe	Bâlteanu *et al.* (2010)
0.37	0.38			0.25			0.55	0.45			Turkish Black & White	Gurcan (2011)
0.24	0.17			0.59			0.32	0.68			Holstein-Friesian	Ren *et al.* (2011)
0.21	0.23			0.56			0.32	0.68			Jersey	Ren *et al.* (2011)

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Table 5.10. Frequency of α-lactalbumin genetic variants.

Genotype			Allele			
AA	**AB**	**BB**	**A**	**B**	**Breed**	**Reference**
		1.00		1.00	Holstein-Friesian	Ng-Kwai-Hang *et al*. (1990)
			0.33	0.67	Gyr Zebu	Da Silva & Del Lama (1997)
			0.30	0.70	Guzerat Zebu	Da Silva & Del Lama (1997)
			0.39	0.61	Sindi Zebu	Da Silva & Del Lama (1997)
			0.18	0.82	Nelore Zebu	Da Silva & Del Lama (1997)
0.28	0.68	0.038	0.62	0.33	Kangayam cattle	Jeichitra *et al*. (2003)
		1.00		1.00	All breeds	Bâlteanu *et al*. (2010)

locus, with a slight predominance of the β-lactoglobulin B allele (Oner & Elmaci, 2006).

Milk protein allele frequencies vary by breed in seven Romanian dairy cattle (Romanian Simmental, Romanian Black and White, Red Holstein, Brown, Black Pinzgau, Red Pinzgau and Grey Steppe). For α_{s2}-casein and α-lactalbumin, the A allele is the only variant in all the studied breeds. In Red Holstein, the α_{s1}-casein B dominated (1.0); however, the C allele was also missing from β-casein, κ-casein and β-lactoglobulin (Bâlteanu *et al*., 2010) (Tables 5.9 and 5.10).

The Holstein group was different from the Jersey breed in a Chinese study, with a higher frequency of the κ-casein A allele and a correspondingly lower frequency of the B allele (Ren *et al*., 2011). Similar results were obtained in Turkey (Gurcan, 2011). At the same time, no AA genotype has been found in Jerseys, where the BB is dominant; on the contrary, the AA genotype has the highest frequency in Holsteins (Ren *et al*., 2011). These results differ from other studies, which have reported the highest frequency for κ-casein B in the Holstein breed (Allmere *et al*., 1998; Oner & Elmaci, 2006) (Table 5.8), however, in other breeds (Da Silva & Del Lama, 1997) the κ-casein A has the higher frequency.

According to the findings of Oner and Elmaci (2006) the α_{s1}-casein B and β-casein A alleles are found very close to fixation limits in the case of Holstein cows, while a similar finding was observed in Jerseys concerning the κ-casein B allele. Because of this, it would be very difficult to change their frequencies.

5.3.2 Effects on milk production

Most of the large-scale studies on the association between milk protein types and production traits have been restricted to either first lactation performance (Ng-Kwai-Hang *et al*., 1984; Lin *et al*., 1986; Gonyon *et al*., 1987; Haenlein *et al*., 1987) or test-day data (Ng-Kwai-Hang *et al*., 1986).

A number of studies have found associations between the other milk protein genotypes and milk yield or gross composition (Aleandri *et al*., 1990), but others have found no effect of milk protein genotype on yield traits (McLean *et al*., 1984). Overall, milk protein genotypes appear to have no consistently significant effect on production traits. This idea is supported by the absence of trends of allelic frequency change in the US dairy cattle population over time (Van Eenennaam & Medrano, 1991).

The phenotypes of α_{s1}-casein have been associated ($P<0.05$) with the milk production of different parities (Ng-Kwai-Hang *et al*., 1990). The B allele is prevalent compared with the C allele (Boettcher *et al*., 2004a, b), while the CC genotype has a stronger positive effect on milk yield and protein content than that of the BC and BB genotypes (Van Eenennaam & Medrano, 1991). Conversely, cows with α_{s1}-casein BB phenotype have higher milk yield than those with AB and BC phenotypes in Turkish Black and White cattle (Gurcan 2011).

The effects of α_{s1}-casein genotypes on milk yield have been reported, although the frequency of these genotypes in the cow population is low and the effects on milk yield not consistent (Lien *et al*., 1995; Prinzenberg *et al*., 2003; Kuss *et al*., 2005; Sanders *et al*., 2006; Kučerová *et al*., 2006).

Variants of β-casein affect milk yield, but the results are not consistent. In earlier works (Ng-Kwai-Hang *et al*., 1984, 1986; Lin *et al*., 1986) the β-casein A allele was reported to be associated with higher milk production. Cows with β-casein A^1A^3 variants are among the highest producers, whereas A^2B cows are among the lowest ones (Ng-Kwai-Hang *et al*., 1990) and the A^3 allele (Ng-Kwai-Hang, 1998) is definitely associated with higher milk yield. According to Çardak (2005), the milk yield of Holstein cows is affected by the β-casein genotypes in a particular order: $A^2A^2 > A^1B > A^2B > A^1A^2 > A^1A^1$. On the other hand, Kučerová *et al*. (2006) stated that the A^1A^1 genotype is associated with highest milk yield in the Czech Fleckvieh breed, while A^2A^2 has a negative effect on yield.

Some studies (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1984; Neubauerová 2001) indicate no significant relationship between milk production and κ-casein

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variants, whereas others (Ng-Kwai-Hang *et al*., 1986; Gonyon *et al*., 1987; Baranyi & Bösze, 2009) have shown differences in milk production depending on the variants of κ-casein, for example the κ-casein BB genotype increases milk yields and protein percentage (Ng-Kwai-Hang *et al*., 1984; Lin *et al*., 1989; Aleandri *et al*., 1990; Van Eenennaam & Medrano, 1991) or κ-casein BB cows produce less milk than κ-casein AA cows (Bovenhuis *et al*., 1992; Neubauerová, 2001; Boettcher *et al*., 2004a, b; Caroli *et al*., 2004; Kučerová *et al*., 2005). According to Çardak (2005), the κ-casein genotypes influenced milk yield equally in the case of Holstein (AA > AB) and Simmental (AB > AA > BB) cows. According to Neubauerová (2001) and others (reviewed by Comin *et al*., 2008 and Caroli *et al*., 2009) the A variant is associated with higher milk yield.

Several contradictory results have been published about the relationship between milk production and β-lactoglobulin genotypes. Some of them (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1984; Lin *et al*., 1986; Gonyon *et al*., 1987; Haenlein *et al*., 1987) have reported no association between β-lactoglobulin genotypes and milk production (Kučerová *et al*., 2006; Botaro *et al*., 2008), while according to others the β-lactoglobulin A cows are higher milk producers (Ng-Kwai-Hang *et al*., 1986; Bovenhuis *et al*., 1992). Baranyi and Bösze (2009) found strong genotype effect on milk yields of various cattle breeds. In various studies, the AA and BB genotype of the β-lactoglobulin gene were associated with higher milk production (Mayer *et al*., 1990; Ng-Kwai-Hang *et al*., 1990; Bovenhuis *et al*., 1992; Hill *et al*., 1996; Ikonen *et al*., 1999). In other reports, the AB genotype resulted in higher milk production (Kaygisiz & Douan, 1999; Tsiaras *et al*., 2005).

Heidari *et al*. (2009) stated that the β-lactoglobulin AA genotype resulted in more milk in Holstein cows than the BB genotype, but the superior milk producers carry the AB genotype. According to Çardak (2005), the β-lactoglobulin (AB > BB > AA) influences the milk yield of Holstein cows, but a different order (AA > AB > BB) was found by Gurcan (2011) in Turkish Black and White cattle. However, the differences were not significantly important.

5.3.3 Effects on milk composition

Fat content and fat yield are not affected by the α_{s1}-casein phenotypes over the course of three lactations for Holstein cows (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1990), while Ng-Kwai-Hang *et al*. (1984, 1986) had concluded earlier that higher milk fat content is found with the BC variant. Protein content is influenced ($P < 0.05$) by phenotypes of α_{s1}-casein during the first two lactations, but not during the third lactation (Ng-Kwai-Hang *et al*., 1990). This confirms the findings of other reports (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1984, 1986; Bovenhuis *et al*., 1992) in which the α_{s1}-casein BC genotype was associated with higher protein in milk than the AB and BB variants.

Cows with β-casein A^1A^1 consistently produce milk with a higher fat content than those with A^1A^2 or A^2A^2 β-casein. Cows with A^2A^2 β-casein produced more milk of lower fat content than cows with A^1A^1 β-casein, but the lactation fat yield is not significantly different between the various phenotypes of β-casein (Ng-Kwai-Hang *et al*., 1990). The β-casein genotypes have significant effects on fat percentage ($P = 0.017$), protein percentage ($P < 0.001$) and protein yield ($P = 0.007$). However, β-casein A^1B and BB cows produce milk with a higher fat and protein content than that of β-casein A^1A^1 cows. The β-casein A^3 is associated with a lower fat content and a higher protein content of milk. The β-casein A^1A^3, A^2A^3 and A^3B cows produce milk with a lower fat content and higher protein content (Bovenhuis *et al*., 1992).

There is a general consensus that the κ-casein B allele is associated with higher protein content (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1984, 1986), and the BB genotype definitely increases protein content and protein quality in milk (Ng-Kwai-Hang *et al*., 1990; Bovenhuis *et al*., 1992; Neubauerová, 2001; Kaminski *et al*., 2002; Boettcher *et al*., 2004a, b; Caroli *et al*., 2004; Kučerová *et al*., 2005), while the protein yield is not necessarily affected by the κ-casein genotypes (Bovenhuis *et al*., 1992).

The κ-casein locus also influences the fat content of the milk. Thus κ-casein BB milk is associated with a higher fat content than that of the AA variant (Ng-Kwai-Hang *et al*., 1986). The effect can be modified by the number of lactations. During the first two lactations no difference is observed between the fat content of κ-casein AA and κ-casein BB milk, but κ-casein AB milk has less fat. The pattern changes during the third lactation: κ-casein AA is associated with the highest and κ-casein BB with the lowest fat content (Ng-Kwai-Hang *et al*., 1990).

The original report of Aschaffenburg and Drewry (1957), showing that β-lactoglobulin AA milk contains more protein than either AB or BB milk, has been confirmed by several workers (Ng-Kwai-Hang *et al*., 1984, 1986). The difference in protein content for various types of β-lactoglobulin is explained by altered rates of synthesis of β-lactoglobulin (McLean *et al*., 1984). The higher protein yield consistently associated with β-lactoglobulin AA is due to higher amounts of whey protein (Ng-Kwai-Hang *et al*., 1990; Bovenhuis *et al*., 1992; Kučerová *et al*., 2005, 2006). The β-lactoglobulin BB genotype is associated with higher casein and fat content, which are favourable for cheesemaking properties (McLean *et al*., 1984; Aleandri *et al*., 1990; Hill, 1993; Lodes *et al*., 1997; Lundén *et al*., 1997; Kaminski *et al*., 2002; Boettcher *et al*., 2004a, b; Caroli *et al*., 2004; Wedholm *et al*., 2006), while higher protein content is also found in

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the case of BB and BE (Kučerová *et al*., 2005) and AB (Kučerová *et al*., 2006) genotypes.

The β-lactoglobulin genotype can also influence the fat content of the milk. Lin *et al*. (1986) and Ng-Kwai-Hang *et al*. (1990) found no relationship between β-lactoglobulin genotypes and fat in milk, while others have shown significant relationships (McLean *et al*., 1984; Ng-Kwai-Hang *et al*., 1984, 1986). Average milk fat was higher in β-lactoglobulin BB milk than in AA milk (Ng-Kwai-Hang *et al*., 1986) during the first two lactations, and the difference was highly significant.

5.3.4 Interactions

Milk yield and especially milk composition are equally affected by milk protein polymorphisms, and they can influence the values together because of several interactions between them. There is general agreement among various authors that the κ-casein genes and the β-lactoglobulin variants have stronger effects on protein yield and content than those of the β-casein and α_{s1}-casein genotypes (Lundén *et al*., 1997; Mayer *et al*., 1997; Robitaille *et al*., 2002; Baranyi & Bösze, 2009; Bonfatti *et al*., 2010). According to Bobe *et al*. (1999) the κ-casein and β-lactoglobulin genotypes affect the genetic and phenotypic variation of milk protein composition and, in particular, the proportions of α_{s1}-casein, κ-casein and β-lactoglobulin in total milk protein, but no significant effect has been observed on milk protein concentration. At the same time, feeding cows diverse diets had only a minimal effect on milk protein composition.

In Simmental cattle (Çardak, 2005), the genotypes of α_{s1}-casein (BC>BB), β-casein ($A^1A^1>A^2A^2>A^1B>A^1A^2>A^2B$), κ-casein (AA>AB=BB) as well as β-lactoglobulin (BB>AA>AB) all equally influenced milk protein content, and there were highly significant differences among the genotypes. Similar results were found by Bonfatti *et al*. (2010), who stated that the genotype BB at β-lactoglobulin and haplotypes carrying β-casein B and κ-casein B were associated with increased casein content and casein number.

Several authors are convinced that because the genes encoding α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein are located on chromosome 6, within a region of about 250 kb (Ferretti *et al*., 1990), it is difficult to assess single casein gene effects (Lien *et al*., 1995) and it is preferable to estimate casein haplotype effects instead of genotype effects (Ikonen *et al*., 2001; Boettcher *et al*., 2004a). Composite casein genotypes (Aleandri *et al*., 1990; Comin *et al*., 2008) and casein haplotypes (Nuyts-Petit *et al*., 1997) were also considered because of the tight genetic linkage among the casein genes.

Among the haplotypes, B-A^1-B (in the order α_{s1}-casein, β-casein, κ-casein) is associated with an increased percentage of both fat and protein in Finnish Ayrshire (Ikonen *et al*., 2001) and Italian Holstein and Brown Swiss cattle, but has negative effects on milk yield (Boettcher *et al*., 2004a). The C-A^2-B haplotype was associated with significantly decreased yield and increased concentration of protein, whereas, in general, haplotypes carrying the κ-casein B allele have positive effects on protein percentage relative to the corresponding haplotypes carrying κ-casein A. In Norwegian Red cattle, Nilsen *et al*. (2009) found highly significant associations with both protein and milk yield within the α_{s1}-casein/β-casein/α_{s2}-casein haplotype block, whereas no significant association was found within the κ-casein block. Heck *et al*. (2009) concluded that selection for the β-casein/κ-casein haplotype A^2B, together with β-lactoglobulin B, would result in Dutch Holstein-Friesian cows that produce milk more suitable for cheesemaking.

5.3.5 Effects on cheesemaking properties

Several authors (McLean *et al*., 1984; Schaar *et al*., 1985; Van Den Berg *et al*., 1992) have reported associations between milk protein genetic variants and the manufacturing properties of milk. Research has focused on the relationship of milk protein loci with cheese production. Two milk protein genes, κ-casein and β-lactoglobulin, have been intensively studied and indicated that κ-casein variants are associated with renneting time, whereas β-lactoglobulin variants are associated with casein number. In both cases, the B variants are favourable.

Cheese-yielding capacity and coagulating properties of milk are influenced by genetic variants of the milk proteins β-lactoglobulin, α_{s1}-casein, β-casein and κ-casein. Rennet clotting time, the rate of curd formation, and coagulum strength are all improved in milk containing the B variant of κ-casein (Schaar, 1984; Schaar *et al*., 1985). This same variant and the β-lactoglobulin B variant are associated with increased cheese yield (Marziali & Ng-Kwai-Hang, 1986; Aleandri *et al*., 1990).

Aleandri *et al*. (1990) found that the differences in Parmesan cheese yield between milk of κ-casein AA and κ-casein BB genotype was greater than that which would have been predicted based on the difference in milk composition alone. Difference in the salted curd yield from milk of β-lactoglobulin genotype AA and BB was also greater than expected; the β-lactoglobulin BB genotype produced a greater than predicted yield. This protein variant effect on cheese yield may be related to the associations between the milk protein genotypes and casein composition. Both the β-lactoglobulin BB and κ-casein BB genotypes are associated with higher casein percentage in milk. The κ-casein variants affect the concentration and proportion of κ-casein (BB>AB>AA), the proportion of α_{s1}-casein, and the concentration of β-lactoglobulin and α-lactalbumin

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(AA>AB>BB). Cows with the β-lactoglobulin AA genotype produce milk with a greater proportion of protein in the form of whey proteins (McLean *et al*., 1984).

The combination of β-lactoglobulin B, β-casein B and κ-casein B would improve the casein content, coagulating properties and cheese-yielding capacity of milk. The α_{s1}-casein genotype has not been shown to have a significant effect on the manufacturing properties of milk (Van Eenennaam & Medrano, 1991).

Comparing the possible haplotypes of α_{s1}-casein/β-casein/κ-casein on cheese yield in the case of Norman cattle, Nuyts-Petit *et al*. (1997) stated that the B-B-B haplotype resulted in the best solution. They found a different decreasing sequence in the case of κ-casein proportion (B-B-B>C-A^2-B>B-A^2-A) and micelle sizes (B-A^2-A>B-B-B>C-A^2-B) in milk. The lowest coagulation ability was achieved with the B-A^2-A haplotype.

In studies of milk from different cattle (Swedish Red and White, Swedish Holstein and Danish Holstein-Friesian), Wedholm *et al*. (2006) and Hallén *et al*. (2008) stated that the concentrations of α_{s1}-casein, β-casein, κ-casein and β-lactoglobulin B had significant effect on cheese yield. The C-terminal fragment of β-casein and several other minor fragments of β-, α_{s1}- and α_{s2}-casein had positive effects on the transfer of protein from milk to cheese (Wedholm *et al*., 2008).

The highest cheese yield could be obtained from milk with genotypes of β-casein A^2B, κ-casein AA and β-lactoglobulin AA. At the same time, the lowest cheese yield might come from milk with genotypes of β-casein A^2A^2, κ-casein AA, β-lactoglobulin AA and the difference could reach 30%. The κ-casein is responsible for the renneting ability of milk, and the κ-casein BB variant gives the best result (Skeie, 2007).

Apart from the fact that cheese is made from caseins, it also contains various amounts of fat, which has a very strong impact on the properties of the cheese and is affected by the protein composition. The lowest fat content of the whey can be expected with protein genotypes β-casein A^2B, κ-casein AA, β-lactoglobulin BB or β-casein A^2A^2, κ-casein AA, β-lactoglobulin BB. Cheese fineness is also affected by the combinations of milk protein genotypes; the lowest amounts of caseins remain in the whey with β-casein A^2B, κ-casein AA, β-lactoglobulin BB or with β-casein A^2B, κ-casein BB, β-lactoglobulin BB (Skeie, 2007).

In Italian Holsteins, Comin *et al*. (2008) found that κ-casein and β-casein were strongly associated with milk coagulation traits and milk and protein yields; the best coagulation time and curd firmness were observed in the case when at least one B allele was available at both the β-casein and κ-casein loci (similar to the earlier findings of DiStasio & Mariani, 2000). Heck *et al*. (2009) concluded that selection for the β-casein/κ-casein haplotype A^2-B, together with β-lactoglobulin B, would result in Dutch Holstein-Friesian cows producing milk more suitable for cheesemaking.

5.3.5.1 β-Lactoglobulin

The β-lactoglobulin genotypes alone can modify the cheese yield properties of milk. Several studies have shown that the BB genotype of β-lactoglobulin is associated with higher fat and increased cheese yields (Ng-Kwai-Hang *et al*., 1984, 1986; Aleandri *et al*., 1990; Hill, 1993; Wedholm *et al*., 2006). Conversely, Bovenhuis *et al*. (1992) reported lower protein yield for this genotype. According to Hill (1993) the milk from β-lactoglobulin AA cows has a 28% higher whey protein concentration, and a 7% lower casein concentration than that of β-lactoglobulin BB cows. The high whey protein concentrates result from the large increase in β-lactoglobulin in milk. The β-lactoglobulin B concentration (Wedholm *et al*., 2006) and the β-lactoglobulin BB genotype (Hallén *et al*., 2008) positively affect cheese yield, whereas β-lactoglobulin A has a negative effect. In addition, the β-lactoglobulin BB genotype increases the milk clotting time (Bonfatti *et al*., 2010) in the milk of Simmental cows as well.

5.3.5.2 κ-Casein

The B allele of both κ-casein and β-lactoglobulin loci may allow for improvement in the quality of milk for manufacturing processes, primarily because milk from cows possessing the B allele at the κ-casein locus is superior for cheesemaking because of faster coagulation and firmer curd, while genotype BB at the β-lactoglobulin locus is associated with higher casein and fat content, also favourable properties for cheesemaking (Aleandri *et al*., 1990; Lundén *et al*., 1997). Other authors have also found that κ-casein loci are related to the quality of milk, and that the B allele is more suited for cheesemaking (Ikonen *et al*., 1999; Patil *et al*., 2003; Azevedo *et al*., 2008). The variants of κ-casein affect casein content, protein content and cheese yield, as well as curd firmness, while β-lactoglobulin is significantly associated with fat, protein, casein, total solids content and cheese yield (Celik, 2003; Hallén *et al*., 2008).

Wedholm *et al*. (2006) stated that the poor and non-coagulating properties of milk are associated with a low concentration of κ-casein and a low proportion of κ-casein in relation to total casein. The κ-casein concentration is higher in milk from cows with the AB genotype than the AA genotype of κ-casein (Skeie, 2007). The increased κ-casein concentration decreases the casein loss in the whey (Hallén *et al*., 2008). In addition to these, the effect of κ-casein B on milk coagulating properties is related to variation in protein composition caused by the allele-

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specific expression of κ-casein, rather than to a direct role of the protein variant on the coagulation process (Bonfatti *et al*., 2010).

Rennet clotting time is favourably affected by κ-casein content and percentage of κ-casein to total casein, whereas curd firmness increases when the content and percentage of β-casein and κ-casein are increased (Bonfatti *et al*., 2010). Decreased content and proportion of β-casein and κ-casein and increased proportions in casein of α_{s1}- and α_{s2}-casein could result in weak curds (Bonfatti *et al*., 2011), while increasing β-casein and κ-casein content and relative proportions in total casein improve curd quality but do not correlate with rennet clotting time.

On the basis of these results, selection for the B allele in the case of both β-lactoglobulin and κ-casein could improve the cheesemaking properties of milk, which confirms earlier findings (McLean *et al*., 1984; Aleandri *et al*., 1990; Lodes *et al*., 1997; Lundén *et al*., 1997; Bonfatti *et al*., 2010).

5.3.5.3 β-*Casein*

Bonfatti *et al*. (2010) found that β-casein B exerted a favourable effect on curd firmness, caused by the increased β-casein content in milk. The relative proportion of β-casein in casein exhibited a genetic correlation (−0.26) with rennet clotting time (Bonfatti *et al*., 2011) and with κ-casein percentage (r=0.44), which is perhaps a contradictory effect.

5.4 MILK PROTEIN VARIANTS AND HUMAN NUTRITION: THE HUMAN BENEFIT

It is noteworthy that milk protein polymorphisms are involved in human nutrition in various ways. Three crucial aspects include the hypoallergenic properties of particular types of milk, the release of peptides with biological functions from milk proteins and the coevolution of bovine milk protein variants and human lactose intolerance (Caroli *et al*., 2009). Since milk protein allergies and lactose intolerance are summarised and evaluated in separate chapters, the bioactive peptides are mainly presented here.

5.4.1 Hypoallergenic milk

Most milk proteins are potential allergens, especially α_{s1}-casein, α_{s2}-casein and β-lactoglobulin, which are lacking in human milk (EFSA, 2004; Crittenden & Bennett, 2005). The occurrence of alleles associated with a null or low content of these proteins might be exploited for the production of milk with particular nutritional qualities, i.e. hypoallergenic properties (Caroli *et al*., 2009).

Besides selecting for milk with a null or reduced content of a specific protein, another possibility for producing hypoallergenic milk could involve genetic differences among epitopes, which are short fragments spread widely throughout the hydrophobic parts of the protein molecules. Epitopes on milk proteins comprise highly conserved sequences responsible for IgE cross-reactivity with corresponding milk proteins of other mammals, including humans (Wal, 2004).

5.4.2 Biopeptides

The two major categories of milk proteins are the insoluble proteins (the casein family) and soluble proteins (whey proteins), found in lactoserum. The casein family of proteins consists of several types of casein (α_{s1}, α_{s2}, β, κ and γ), while the whey proteins are α-lactalbumin and β-lactoglobulin. Milk also contains important minor proteins, such as serum albumin, immunoglobulins, lactoferrin, transferrin, calcium-binding protein, prolactin, folate-binding protein and protease-peptone (Park *et al*., 2007).

In recent years, it has been recognised that dietary proteins provide a rich source of biologically active peptides. Biopeptides have been defined as specific protein fragments that have a positive effect on body functions or conditions and might ultimately influence health (Kitts & Weiler, 2003). Such peptides are inactive within the sequence of the parent protein but can be released from precursor proteins by enzymatic proteolysis during gastrointestinal digestion or food processing (Fitzgerald & Murray, 2006; Korhonen & Pihlanto, 2006; Korhonen, 2009). Caseins represent a reservoir of a wide variety of bioactive peptides, i.e. minor regulatory compounds with hormone-like activity, which could affect the nutritional value of milk (Meisel, 1998; Lorenzini *et al*., 2007; Korhonen, 2009). There are several effects of biopeptides in the human body (Cozma *et al*., (2011):

- In the gastrointestinal system: regulation of mineral absorption, anorexigen activity (Clare & Swaisgood, 2000; Korhonen & Pihlanto, 2006).
- In the cardiovascular system: antihypertensive, antithrombotic, hypocholesterolaemic and antioxidant activities (Fitzgerald *et al*., 2004; Silva & Malcata, 2005; Korhonen & Pihlanto, 2006; Pihlanto, 2006).
- In the nervous system: agonist and antagonist opioid activities (Silva & Malcata, 2005; Korhonen & Pihlanto, 2006).
- In the immune system: antimicrobial, immune-modulatory and cytomodulatory activities (Korhonen & Pihlanto, 2006; Park *et al*., 2007).

Once absorbed, casein peptides have the potential to exert numerous biological effects in the human, i.e. they may play a crucial role in the transport and absorption of certain

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minerals, bind toxins, mediate immunomodulatory effects and behave as opioid receptor agonists or antagonists (Phelan *et al*., 2009). Because of their biological activity, serious interest has also been raised regarding whey proteins that exhibit a diverse array of non-specific activities, i.e. anti-inflammatory, bacteriostatic, antioxidant, opioid and anticancer properties (Chatterton *et al*., 2006; Pan *et al*., 2006).

El-Agamy (2007) has reviewed cow milk protein allergy and found that about 0.3–7.5% of the population could be affected, determined by country and society, and ethnicity; the allergy occurs predominantly in children and is only seen occasionally in adults. Most of the summarised studies state that β-lactoglobulin is the most allergenic protein in bovine milk, as it is lacking in human milk, but α_{s1}-casein as well as β-casein and α-lactalbumin, bovine serum albumin and IgG could also cause allergy. Most of these proteins are the source of various peptides liberated during digestion or during milk processing.

There have been several studies on the peptides originating from β-casein, and special attention has been given to the A^1, B and A^2 variants. Proteolytic digestion of bovine β-casein variants A^1 and B leads to the release of the bioactive opioid peptide β-casomorphin 7 (BCM-7), but this was not seen with variant A^2. An amino acid substitution at position 67, with histidine replacing proline, is responsible for the production of BCM-7, which has been branded a dangerous peptide. Eight different casomorphins have been detected so far. Many reports have stated that this substitution may be responsible for several diseases, and the milk from cows with A^1 or B variants is called 'histidine carrier'. Elliott *et al*. (1999) have suggested that consumption of milk from His_{67}-carrying cows is positively and significantly correlated with the incidence of diabetes. It has been stated in other work (Beaglehole & Jackson, 2003; Laugesen & Elliott, 2003) that the A^1 allele increases the risk of diseases such as diabetes, coronary heart disease and ischaemic heart disease in people consuming such milk. On the other hand, these authors did not find any negative effect of the A^2 allele.

According to Molkhou (2006), α_{s1}-casein has been incriminated as the major milk allergen. Similar results were published by Kost *et al*. (2009) who stated that the β-casein A^1, B and C genetic variants definitely led to the above-mentioned diseases, but that digestion of β-casein A^2 and A^3 variants had no adverse influence. Finding almost the same results, Nilsen *et al*. (2009) suggested using selection breeding in order to increase the frequency of alleles or haplotypes coding for Pro_{67} at the β-casein locus. Moreover, in New Zealand, a new 'industrial trend' has been initiated (FSANZ, 2007) in order to produce more desirable milk from cows carrying only the A^2 variant of β-casein, and the name of the company which produces it is called the A^2 Corporation. However, this positive effect of allele A^2 is not fully consistent with its effect on milk production parameters.

In order to clarify this situation regarding the different opioid properties of milk peptides, including BCM-7, an important study was carried out by the European Food Safety Authority (EFSA). According to this scientific report (EFSA, 2009) almost all bovine milk proteins (α-casein, β-casein, κ-casein, β-lactoglobulin, α-lactalbumin, lactoferrin) could be the source of opioid ligands/peptides, and all of these were evaluated. From among the eight β-casomorphins, BCM-7 has been suggested to be the most problematic in humans according to many authors.

In the official report, covering almost the last 40 years, it was stated that the β-caseins of various breeds of cattle from several countries showed very high breed and country (environment) differences. The genotypes A^1 and A^2 seemed to be dominant, while the frequency of other variants (A^3, B and C) was very low. The dominance of the A^1 or A^2 variant is quite unbalanced. In some breeds (Ayrshire, Black Pied, Dutch Friesian, most Holsteins and Red Danish) the A^1 variant dominates, while in others (Jersey, Finn cattle, Brown Italian, Brown Swiss, Simmental and the remaining Holsteins) the A^2 variant allele has the higher frequency.

Based on a final evaluation of the results of many scientific reports, the authors stated that caution is needed because of conflicting results about the potential health effect of β-casomorphins and related peptides. In particular, a cause–effect relationship between the oral intake of BCM-7 and the aetiology of any suggested diseases cannot be established, and consequently a formal EFSA risk assessment of food-derived peptides is not recommended.

Investigations into other genetic polymorphisms potentially affecting milk protein peptides are scarce (reviewed by Caroli *et al*., 2009). Weimann *et al*. (2009) studied the peptides derived from the genetic variants κ-casein A, B, C, E, F^1, F^2, G^1, G^2, H and I for their antihypertensive activities. The AA sequences of the κ-casein variants were analysed *in silico* to detect potential inhibitory peptides against angiotensin I-converting enzyme. Some κ-casein variants carried the following exclusive peptides whose angiotensin I-converting enzyme inhibitory activity was determined: ASP (within κ-casein B), AHHP (κ-casein C), VSP (κ-casein F^1) and ACHP (κ-casein G^2). Tulipano *et al*. (2010) investigated the effects of four selected casein peptides on osteoblast mineralisation *in vitro*. The peptides were related to different casein genetic variants, in particular β-casein C and α_{s2}-casein C versus the other β-casein and α_{s2}-casein variants, respectively.

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5.5 THE MINOR PROTEINS

Milk contains numerous minor proteins, some of which are associated with casein fractions, enzymes or fat globule membranes (Groves, 1971) or complexes with metal ions, or vitamins, and these are predominantly found in the whey (Fox & Flynn, 1992; Haggarty, 2002). When ways of increasing the value of milk proteins are discussed, the focus is usually on these minor proteins but they are, in fact, of little economic value to the overall dairy industry (Fox & Kelly, 2003).

In general, there are more than 200 minor proteins in milk of which about 60 are endogenous enzymes. Most of the minor proteins have biological functions (Fox & McSweeney, 1998) and probably play quite significant roles. Important minor proteins include immunoglobulins, lactoferrin, transferrin, ferritin, proteose peptone, calmodulin (calcium-binding protein), prolactin (50 µg/mL in cows' milk; Park *et al*., 2007) and folate-binding protein. Whey proteins also include a long list of enzymes, hormones, growth factors, nutrient transporters and disease resistance factors.

In the last couple of years the real values of more and more minor proteins have been determined. Smolenski *et al*. (2007) identified 53 minor proteins using liquid chromatography–mass spectrometry that belonged to eight functional categories: host defence/immune related (19%), enzyme (13%), structural (17%), transport (15%), DNA binding (11%) and signal transduction proteins (11%), as well as 13% that were of unknown or unclassified function. Utilising two-dimensional electrophoresis, they managed to identify and assign 57 minor proteins; from among the most important ones were the structural proteins (26%), enzymes (6%), transport proteins (14%), host defence/immune-related proteins (14%) and chaperone proteins (10%, only in the case of mastitic milk).

5.5.1 Lactoferrin

Certain closely related proteins have the unique ability to bind iron at alkaline pH, producing a salmon-red complex in solution. These belong to a family of proteins called transferrins that are present in blood and other exocrine secretions such as milk, saliva, tears and nasal secretions. The iron-binding protein found in milk and colostrum but absent in blood is called lactoferrin (Groves, 1971). It is a soluble glycoprotein that binds two Fe(III) ions per molecule. Lactoferrin exhibits antimicrobial, antiviral, antioxidant, anti-inflammatory, immunoregulatory and cancer-preventing properties. Its iron-binding properties allow it to deprive bacteria of an element essential for their growth (Anon., 2003). It is thought to be responsible for iron absorption and bioavailability (Riechel *et al*., 1998; Korhonen, 2009).

According to several studies published on the lactoferrin content of bovine milk, levels of transferrin and lactoferrin vary within quite a wide range, from 2 to 20 mg/dL, respectively (Park *et al*., 2007); however, these levels are substantially lower (i.e. by about 1 mg/mL and 0.2 mg/mL in colostrum and milk, respectively) compared with human milk (2 mg/mL lactoferrin) (Fox & McSweeney, 1998; Farrell *et al*., 2004; Park *et al*., 2007). Others have found much higher values of lactoferrin, and observed significant breed differences, as well as seasonal effects on the levels in various breeds (56.10–164.12 µg/mL, Riechel *et al*., 1998; 70–110 mg/L, Lindmark-Månsson *et al*., 2003; 1.11–1.37 g/L, Reklewska *et al*., 2003; 91.4–128.7 mg/L, Król *et al*., 2010).

5.5.2 Serum albumin (bovine serum albumin)

BSA is not synthesised in the mammary gland, but appears instead in milk following passive leakage from the bloodstream. It is the most abundant protein in the circulatory system and constitutes around 50% of the protein in bovine blood. It is a multifunctional protein and is mainly responsible for the maintenance of blood pH, but also acts as an excellent protein reserve and functions as an important transport protein for a wide variety of ligands, including long-chain fatty acids, steroid hormones, bilirubin and metal ions (Carter & Ho, 1994). Because of its size and complex structure, BSA can bind to free fatty acids and other lipids, as well as flavour compounds (Kinsella *et al*., 1989).

The amount of BSA in bovine milk is highest on the day of parturition, and it is found in the fraction rich in α-lactalbumin and β-lactoglobulin (Groves, 1971). Walstra and Jenness (1984) found a BSA content of 1.2% (0.4 g/L). This represents about 1.5% of total milk protein and about 8% of total whey protein (Farrell *et al*., 2004). This low level probably means that it has a very small physiological or technological significance (Fox & Kelly, 2003). In a review, Madureira *et al*. (2007) found a wide range of BSA values (0.02–0.35 mg/mL). According to the latest results (Król *et al*., 2010), the BSA content of milk (0.41–0.47 g/L) is significantly affected by the breed of the cow. At the same time, a seasonal effect is also detectable on BSA values in several breeds.

5.5.3 Immunoglobulins

Immunoglobulin content can reach 0.7 g/L (Walstra & Jenness, 1984) or 2.1% of the total protein in mature milk, while in colostrum this ratio might approach 10% (Fox & Kelly, 2003), and comprise 6% of total whey protein (Table 5.11). The principal immunoglobulin in milk is IgG1, with lesser amounts of IgG2, IgG3, IgA and IgM (Fox & Kelly, 2003).

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Table 5.11. Immunoglobulin content of milk.

Globulin	Csapó & Csapóné (2002)	Farrell *et al.* (2004)	Park *et al.* (2007)
IgG			590 μg/mL
IgG1	1.2–3.3%	0.3–0.6 g/L	
IgG2	0.2–0.7%	0.05 g/L	
IgA	0.2–0.7%	0.01 g/L	140 μg/mL
IgM	0.1–0.7%	0.09 g/L	50 μg/mL

In the colostrum of cow milk, IgG predominates, forming about 80–90% of total immunoglobulins, while the proportion of IgM is about 7% and IgA 5%. IgG1 accounts for 80–90% of IgG, IgG2 for 10–20% (Renner *et al*., 1989). The level of all isotypes found in colostrum (IgA 3.9 mg/mL, IgG 47.6 mg/mL, IgM 4.2 mg/mL) diminishes rapidly after parturition (Park *et al*., 2007), whereas IgG predominates also in mature milk (Table 5.11). At the same time, the IgG level can vary between 10 and 50 mg/mL and increases with parity, from 29.05 mg/mL (1) to 31.59 mg/mL (3) (Ahn *et al*., 2006).

5.5.4 Hormones

Milk contains several protein hormones at trace levels, including epidermal growth factor, insulin, insulin-like growth factor (IGF)-I and IGF-II and mammary-derived growth factor, but the function of these molecules in milk is not known (Fox & Flynn, 1992).

5.5.5 Growth factors

According to a review by Korhonen (2009) the following growth factors have been identified in bovine mammary secretions: β-cellulin (BTC), epidermal growth factor (EGF), fibroblast growth factor (FGF)-1 and FGF-2, IGF-I and IGF-II, transforming growth factor (TGF)-β1 and TGF-β2, and platelet-derived growth factor (PDGF). The concentrations of all known growth factors are highest in colostrum during the first hours after calving and decrease substantially thereafter.

5.5.6 Milk enzymes

Milk contains around 60 endogenous enzymes (e.g. plasmin, lipoprotein lipase, alkaline phosphatase), many of which are significant in various aspects of dairy technology for the stability and quality of milk and dairy products. These molecules can also be used as indices of the thermal history of milk and of mastitis, and may function as protective and digestive aids (Fox & Kelly, 2003).

5.5.6.1 Lysozyme

Lysozyme is a relatively small basic protein and is classified as 1,4-β-*N*-acetylmuramidase, which hydrolyses glycosidic bonds in Gram-positive bacterial walls. The amount in bovine milk is about 13 μg/dL (Bank & Tranter, 1986). While bovine milk normally contains very low levels of lysozyme (0.1 μg/mL), mastitic milk contains higher concentrations (1–2 μg/mL). According to Mullan (2003) lysozyme is one of the major antimicrobial proteins in milk.

5.5.6.2 Lactoperoxidase

Lactoperoxidase is a basic glycoprotein that contains one haem group. As an enzyme, it has an iron content of 0.068–0.071% and a carbohydrate content of 9.9–10.2% (Carlström, 1969). Lactoperoxidase is one of the major antimicrobial proteins in milk (Mullan, 2003) and occurs naturally in colostrum, milk and many other human and animal secretions. Lactoperoxidase represents the most abundant enzyme in milk (Korhonen, 2009).

5.5.7 Metal-binding proteins

Milk contains several metal-binding proteins, of which the caseins are quantitatively the most important. Several enzymes are metalloproteins, e.g. xanthine oxidase (Fe, Mo), alkaline phosphatase (Zn, Mg), lactoperoxidase (Fe), catalase (Fe) and glutathione peroxidase (Se). The most important metalloprotein is lactoferrin, which includes transferrin and ovotransferrin (conalbumin). It is not milk-specific, being present in several body fluids (Fox & Kelly, 2003).

5.5.8 Vitamin-binding proteins

The specific vitamin-binding proteins are nutritionally significant (Fox & Flynn, 1992; Haggarty, 2002). Most folate and its derivatives in raw bovine milk are bound to a folate-binding protein, which is present at a concentration of 10 mg/L (Fox & Kelly, 2003). However, Park *et al*. (2007) found a slightly lower value (8 μg/mL).

A vitamin D-binding protein (DBP) has high structural homology with BSA and can bind long-chain fatty acids. DBP has been detected in milk at much lower levels (2–9%) than in blood serum. The concentration of DBP is higher in bovine colostrum and early milk than in mature milk, and the protein is probably derived from blood serum (Fox & Kelly, 2003).

Three proteins are required for the uptake of vitamin B_{12} (cobalamin) in mammals. Gastric intrinsic factor binds the free vitamin and transfers it to transcobalamin (TC). The TC–cobalamin complex and unsaturated TC are released into the portal plasma along with the third cobalamin-binding protein, haptocorrin, the function of which in plasma is not clear (Fox & Kelly, 2003).

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Riboflavin-binding protein is in complex with riboflavin, and has good antioxidant properties. The riboflavin-binding protein in milk may be derived from serum; its physiological function has not yet been determined (Fox & Kelly, 2003).

5.5.9 Glycoproteins

Several minor glycoproteins have been found in milk and colostrum, but their identity and function have not been fully elucidated (Fox & Kelly, 2003).

5.5.10 Lactollin

A crystalline protein, lactollin, has been found to be associated with the red protein in very small amounts when the red protein is isolated from bovine milk. It appears to be present in colostrum in a significantly higher amount than in normal milk (Groves, 1971).

5.5.11 β_2-Microglobulin

The β_2-microglobulin of bovine milk was first isolated from the red protein fraction. It occurs freely in body fluids and on the surface of all nucleated cells. It is produced in milk by proteolysis of the cellular fraction of milk, mainly during storage within the mammary gland. No significance has been attached to β_2-microglobulin in milk (Fox & Kelly, 2003).

5.5.12 Osteopontin

This protein is found in bone and many normal and malignant tissues as well as in milk and urine. It can bind to many cell types, and has a diverse range of functions (Denhardt & Guo, 1993; Bayless *et al*., 1997), but its role in milk is not clear. It may be important owing to its calcium-binding ability or putative anti-infectious activity (Fox & Kelly, 2003).

5.5.13 Proteose peptone 3

Bovine proteose peptone 3 (PP3, also called lactophorin) is an endogenous milk protein synthesised in the mammary gland and found at a concentration of 0.8 g/L and 2.4% of total protein (Walstra & Jenness, 1984). Initially, PP3 was considered to be exclusively a whey protein but it is also present in the fat globule membrane (Fox & Kelly, 2003).

5.5.14 Milk fat globule membrane proteins

The milk fat globule membrane (MFGM) proteins play an important role in various cellular processes and defence mechanisms in the newborn (Cavaletto *et al*., 2008); they account for only 1–4% of total milk protein (Vanderghem *et al*., 2010). Depending on the source, MFGM is composed of 25–60% protein and the mass of fat globules accounts for 2–6% of the total mass (Singh, 2006).

5.6 NON-PROTEIN NITROGEN

While early reports (Davis, 1952; Armstrong, 1959) ignored the 5–6% of NPN content of milk (DePeters & Ferguson, 1992), with strong breed and herd effects, Jenness and Patton (1959) reported a 'notable resemblance' between the compounds found in the NPN fraction of milk and those found in the urine of cows, suggesting that most of the NPN compounds are end products of nitrogen metabolism. These end products (NPN) could either be derived from blood or be the result of the degradation of milk proteins (Kuzdzal-Savoie *et al*., 1980).

Several factors influence the NPN level in milk, including management, feed and diet, feeding practices, the season, herd and breed, as well as the stage of lactation (Packard, 1984; Wood & Boettcher, 2003; Mech *et al*., 2008; Meehl *et al*., 2010) or the time of the day (Meehl *et al*., 2010). However, the significance of this milk nitrogen fraction to energy and nitrogen metabolism in the dairy cow has not been well characterised. Blood urea nitrogen (BUN) has been positively associated with the intake of ruminally degradable and undegradable protein and negatively associated with the intake of net energy (DePeters *et al*., 1993).

NPN in milk is probably the least understood nitrogen fraction: it has little nutritional value and does not contribute to cheese yield (Packard, 1984; DePeters *et al*., 1993; Barbano & Lynch, 1999). Therefore, it does not have the same economic value as 'true' milk protein to either the processor or the consumer (Barbano & Lynch, 1999). However, NPN is a normal part of the milk, comprising 5–6% of total milk nitrogen, in a very heterogeneous fraction. This group of substances is not protein in nature, but includes many different variants which are present in the aqueous solution of milk.

Alston-Mills (1995) evaluated the data in the literature and determined the average NPN concentrations in bovine milk: total NPN 296.4 mg/L; urea 142.1 mg/L; creatine N 25.5 mg/L; creatinine N 12.1 mg/L; uric acid N 7.8 mg/L; orotic acid 14.6 mg/L; hippuric acid N 4.4 mg/L; peptide N 32.0 mg/L; ammonia N 8.8 mg/L; α-amino acid 44.3 mg/L. Several amines (e.g. choline), amino acid derivatives (e.g. histamine, taurine) and other compounds (e.g. carnitine, morphine) are also found in bovine milk.

In their summary, Fox and McSweeney (1998) reported slightly different concentrations of these compounds in milk: ammonia 6.7 mg/L; urea 83.8 mg/L; creatinine 4.9 mg/L; creatine 39.3 mg/L; uric acid 22.8 mg/L; α-amino nitrogen 37.4 mg/L; and unaccounted N 88.1 mg/L. However, they noted that the latter included some phospholipids, amino sugars, nucleotides, hippuric acid and orotic acid, while the α-amino nitrogen could also contain free amino acids, small peptides and almost the whole range of

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amino acids. They are all present in the blood from where they are transferred to the milk. Stating the dominant role of urea in the NPN fraction, Barbano and Lynch (1999) found other low-molecular-weight nitrogen-containing compounds such as creatine and creatinine as well.

Free amino acids, representing 10–20% of the NPN in milk, are present at a concentration of 5–8 mg/dL (Renner *et al*., 1989). Free amino acids mainly consist of the non-essential amino acids like glutamic acid, glycine, aspartic acid and alanine, while the other amino acids are present as free amino acids only in very low concentrations (Renner *et al*., 1989). Taurine and carnitine do not occur as protein-bound amino acids, but they have essential physiological functions in the newborn. Normal milk contains about 0.6 mg/mL and bovine colostrum 8 mg/dL of taurine. Bovine milk has six to seven times higher carnitine content (160–270 mmol/mL) than human milk.

5.6.1 Urea

The blood is the major source of NPN in cow milk. Milk urea nitrogen (MUN) is probably derived primarily from BUN because urea equilibrates with body water. This equilibration accounts for the high correlation between MUN and BUN (Oltner & Wiktorsson, 1983; Oltner *et al*., 1985; Veen & Bakker, 1988; Martinez *et al*., 1991). Other sources of MUN exist, for example in arginine catabolism in the mammary gland (Annison, 1983), but they are likely to be less important. The nitrogen in BUN can be derived from at least two sources: the digestion of nitrogenous compounds within the gastrointestinal tract or amino acid catabolism in the liver. These two sources have been briefly addressed in relation to diet (DePeters *et al*., 1993).

The dominant component of milk NPN is urea, accounting for 50% of the nitrogen content in general, although Kaufmann (1982) reported that urea may constitute 20–75% of the NPN fraction while Hojman *et al*. (2004) calculated it as 36.1–38.4%. It is an important parameter of milk quality and is an indicator of the adequacy of herd nutritional practices. Large values of urea content indicate an unbalanced ratio of protein versus energy in the feed and inefficient utilisation of protein. Additionally, the milk urea content affects reproduction (Godden *et al*., 2001; Mitchell *et al*., 2005). Recent studies on urea and casein contents indicate that variability among cows exists (Wood *et al*., 2003; Ikonen *et al*., 2004; Mitchell *et al*., 2005).

The values of milk MUN could be changed by numerous factors, so the data published from around the world are different. When evaluating the seasonal effect on MUN, there is no agreement concerning the highest values. When different breeds or various herds are included in the studies, strong breed or herd effects are observed in the values for MUN (Arunvipas *et al*., 2003; Wood & Boettcher, 2003; Hojman *et al*., 2004; Jílek *et al*., 2006; Botaro *et al*., 2008; Bendelja *et al*., 2011; Jonkus & Paura, 2011). Other studies on urea and casein content indicate that variability among cows exists (Wood *et al*., 2003; Ikonen *et al*., 2004; Mitchell *et al*., 2005).

Apart from the herd effect, milking techniques could also affect MUN values (Jonkus & Paura, 2011), as automatic systems induce higher levels compared with side-by-side milking. In most cases, MUN values increase with the milk yield (Godden *et al*., 2001; Arunvipas *et al*., 2003; Hojman *et al*., 2004; Jílek *et al*., 2006; Konjačić *et al*., 2010), whereas others have reported a negative relationship (Ismail *et al*., 1996; Trevaskis & Fulkerson, 1999). The cow's age (Trevaskis & Fulkerson, 1999; Wood *et al*., 2003) affected the MUN content of the milk, but others (Eicher *et al*., 1999) found no age correlation between MUN and milk production. Several authors have observed a variable effect of parity on MUN values (Jílek *et al*., 2006; Konjačić *et al*., 2010; Bendelja *et al*., 2011; Jonkus & Paura, 2011), while others (Eicher *et al*., 1999; Ahn *et al*., 2006) have found no real correlation with parity; however, the age of the cow does have an effect on the value of MUN (Wood & Boettcher, 2003; Hojman *et al*., 2004). Additionally, milk urea content affects reproduction (Godden *et al*., 2001; Mitchell *et al*., 2005).

Findings regarding the effect of lactation stage on MUN values are not consistent. An increasing trend has been found (Ng-Kwai-Hang *et al*., 1985; Trevaskis & Fulkerson, 1999; Hojman *et al*., 2004), as well as reduced levels at the end of the first third of lactation (Arunvipas *et al*., 2003; Ahn *et al*., 2006). High levels were also found in mid-lactation (Konjačić *et al*., 2010; Jonkus & Paura, 2011), but Schepers and Meijer (1998) found no variation with stage of lactation. At the same time, the time of day has a strong effect on the values of MUN (Meehl *et al*., 2010; Bendelja *et al*., 2011) independent of breed.

It is obvious that the available feed and the level of dietary crude protein have a strong effect on MUN (Ropstad *et al*., 1989; Wood & Boettcher, 2003; Hojman *et al*., 2004; Konjačić *et al*., 2010; Meehl *et al*., 2010), but in relation to total milk protein (Hojman *et al*., 2004) and the percentage of milk fat (Jílek *et al*., 2006; Konjačić *et al*., 2010) these levels have negative relation to MUN. However, Hojman *et al*. (2004) observed a positive correlation between MUN and the fat content in milk. Apart from these effects, the influence of non-nutritional factors on milk urea concentration amounts to 13.3%, and the influence of milk production and environment explains 37% of the variation (Jílek *et al*., 2006).

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6
Milk Protein Allergy

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6.1 INTRODUCTION

Milk is certainly one of the most nutritious foods in the human diet. However, for some consumers, milk causes adverse reactions. These adverse reactions can range from lactose intolerance to allergic reactions provoked by various milk proteins. This chapter focuses on milk protein allergy. Many different types of individualistic adverse reactions to foods are known to occur to dietary components (Box 6.1) (Eigenmann, 2007). A food allergy is a reproducible adverse health effect mediated by the immune system that occurs on exposure to a particular food (Johansson *et al*., 2004; Taylor & Hefle, 2006; Boyce *et al*., 2010). Typically, the immune response is directed at specific proteins (known as allergens) that are naturally present in the food in question (Taylor & Hefle, 2006). Food allergies can be classified into several categories based on their immunological mechanism, including IgE-mediated, non-IgE-mediated, and mixed IgE- and non-IgE-mediated allergies (Taylor & Hefle, 2006; Boyce *et al*., 2010). Milk can be involved in all these allergic mechanisms, although IgE-mediated milk allergy is the best studied and most thoroughly understood.

6.2 IgE-MEDIATED FOOD ALLERGY

6.2.1 Mechanism

IgE-mediated allergy, also referred to as immediate hypersensitivity, is characterized by the production of IgE antibodies to specific proteins in an allergenic food. These antibodies then mediate the allergic reactions, which occur within minutes of ingesting the offending food (Taylor & Hefle, 2006). The pathophysiological mechanism for IgE-mediated allergy involves two crucial phases: sensitization and reaction elicitation (Fig. 6.1). During the sensitization phase, an individual is exposed to a food and his or her immune system produces allergen-specific IgE. These antibodies are then attached to the surface of mast cells and/or basophils. On subsequent exposure to the allergenic food, the allergen cross-links specific IgE antibodies on the surface of the mast cell or basophil, which leads to the release of physiologically active mediator molecules

Box 6.1. Classification of individualistic adverse reactions to milk

True food allergies

Antibody-mediated milk allergies (immediate hypersensitivity)
- IgE-mediated milk allergy including oral allergy syndrome
- Exercise-associated milk allergy (milk+exercise)

Cell-mediated food allergies (delayed hypersensitivity)
- Milk protein-induced enterocolitis
- Milk protein-induced enteropathy
- Milk protein-induced proctitis

Either antibody-mediated and/or cell-mediated
- Allergic eosinophilic gastroenteritis
- Allergic eosinophilic esophagitis

Food intolerances

Metabolic food disorders: lactose intolerance

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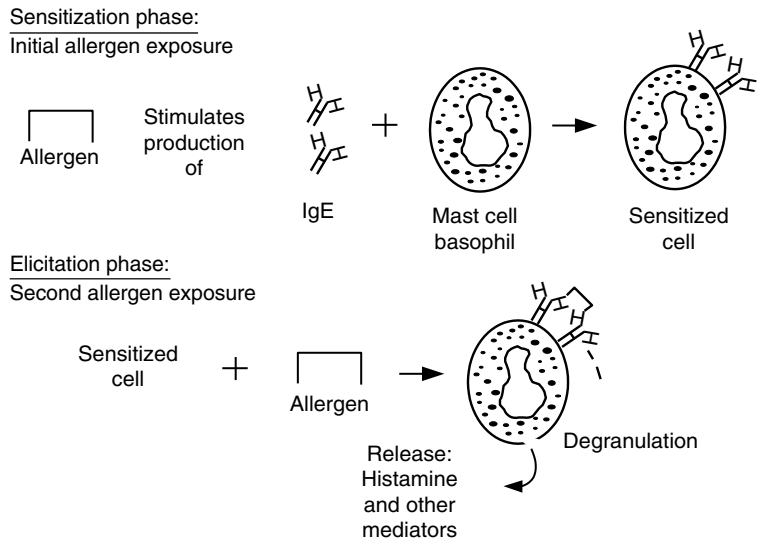


Figure 6.1. Mechanism of IgE-mediated allergic reaction: sensitization followed by elicitation.

(e.g. histamine, leukotrienes, and prostaglandins). The mediators act on various tissues to induce the symptoms of an allergic reaction (Taylor & Hefle, 2006).

The symptoms of an allergic reaction can be quite varied (Box 6.2) and include gastrointestinal (oral allergy syndrome, nausea, vomiting, diarrhea, abdominal pain), cutaneous (urticaria, atopic dermatitis, angioedema, pruritus), respiratory (rhinitis, asthma, laryngeal edema), and generalized symptoms (anaphylactic shock) (Taylor & Hefle, 2006). Systemic reactions involving symptoms beyond the oral cavity and gastrointestinal tract are referred to as anaphylaxis. While all cases of anaphylaxis are not classified as severe reactions, many are. In the USA, food-induced allergic reactions account for approximately 125 000 emergency department visits, 2000 hospitalizations, and 150–200 deaths per year. In addition, food allergic reactions are the leading cause of anaphylaxis, with an estimated 50 000 incidents per year, accounting for 36% of all anaphylaxis cases (Yocum *et al*., 1999; Sampson, 2003; Ross *et al*., 2008; Branum & Lukacs, 2009; Clark *et al*., 2011).

Box 6.2. Symptoms associated with IgE-mediated milk allergy

Gastrointestinal
Nausea
Vomiting
Diarrhea
Abdominal cramping
Oral allergy syndrome

Cutaneous
Urticaria
Dermatitis or eczema
Angioedema
Pruritis

Respiratory
Rhinitis
Rhinoconjunctivitis
Asthma
Laryngeal edema
Heiner's syndrome

Generalized
Anaphylactic shock

6.2.2 Commonly allergenic foods

The medical literature contains reports of allergic reactions to more than 160 foods (Hefle *et al*., 1996). While eight foods account for approximately 90% of food allergies, i.e. peanut, tree nut, soybean, milk, egg, fish, crustacean shellfish, and wheat (FAO, 1995), various countries have established lists of priority (common) allergenic foods that are consistent in listing these eight foods but variable in their listing of other foods (Table 6.1). In order to assure that allergic consumers have the most accurate information about the contents of a food product, several regulatory bodies in the USA, Canada, and Europe require food manufacturers to

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Table 6.1. Priority allergenic foods for selected countries.

Allergenic food	USA	Canada	European Union	Australia/ New Zealand
Cows' milk	✓	✓	✓	✓
Egg	✓	✓	✓	✓
Crustacean shellfish	✓	✓	✓	✓
Fish	✓	✓	✓	✓
Peanut	✓	✓	✓	✓
Tree nuts	✓	✓	✓	✓
Soybean	✓	✓	✓	✓
Wheat	✓	✓	✓	✓
Sesame seed		✓	✓	✓
Molluscan shellfish		✓	✓	
Celery			✓	
Lupine			✓	
Mustard		✓	✓	

indicate the presence of these eight allergenic foods on product labels (United States Congress, 2004; Commission of the European Communities, 2003; Canadian Food Inspection Agency, 2008). In addition to these eight foods, Canada requires sesame seeds, molluscan shellfish, and mustard to be labeled, and the EU requires labeling of celery, mustard, sesame seeds, lupine, and molluscan shellfish (Commission of the European Communities, 2003; Canadian Food Inspection Agency, 2008).

6.2.3 Sensitization and its prevention

The exact reasons for a particular individual developing a food allergy remain unclear. However, a number of risk factors have been identified. Development of food allergy is strongly influenced by genetic factors. For example, individuals are at an increased risk of developing a specific food allergy when a family history of that allergy is present (Lack *et al*., 2003). Monozygotic twins display a significantly higher rate of food allergy than do dizygotic twins, indicating a strong genetic influence (Sicherer *et al*., 2000). Researchers have also found that individuals with asthma and hay fever have a higher prevalence of food allergies (Liu *et al*., 2010), indicating that allergies to any cause are a risk factor beyond allergy to a specific food. Other environmental factors, including environmental exposure to food proteins from cutaneous or respiratory exposure, may also be risk factors for the development of food allergies (Lack *et al*., 2003).

Cows' milk allergy particularly affects infants because cows' milk is introduced very early into the diets of infants who are not breast-fed. Preventing the development of food allergies, including milk allergy, is a somewhat controversial topic. Much debate exists between infant feeding patterns and the later development of food allergies. At one time, the American Academy of Pediatrics recommended that parents delay feeding solid foods until 6 months of age, with further delay of cows' milk until after the first birthday, eggs until the age of 2, or peanuts, nuts and fish until the age of 3 (American Academy of Pediatrics Committee on Nutrition, 2000). These recommendations were reversed in 2008 after studies showed no good evidence that this advice lowered the risk of developing food allergies in children. In addition, evidence is lacking that maternal dietary restrictions during pregnancy and lactation prevent atopic disease (Greer *et al*., 2008).

Food allergy guidelines released by the National Institute of Allergy and Infectious Diseases makes consensus recommendations of breastfeeding for the first 4–6 months of life and not avoiding allergenic foods as a way to prevent allergy. The guidelines also conclude that mothers should not eliminate foods, either during pregnancy or during breastfeeding, in an attempt to prevent food allergy (Boyce *et al*., 2010).

Breastfeeding is the optimal nutrition for all infants. Health effects of breastfeeding are well documented and apply to both mother and infant. Breastfeeding has been associated with a reduced risk of many diseases in neonates, including otitis media, eczema, gastrointestinal infection, asthma, obesity, and some cancers (US Department of Health and Human Services, 2011). Studies examining the effect of breastfeeding on the prevention of food allergy are limited mainly because of methodological logistics. Because it is unethical to randomize breastfeeding against formula feeding, the preventive effect is unknown and requires long-term studies. Existing studies are limited to being observational, nonrandomized, or retrospective and are confounding and biased. Systematic reviews and meta-analyses of existing studies show conflicting results, some showing a protective effect, no effect, or even increased risk (Grimshaw *et al*., 2009; Kneepkens & Brand, 2010). Regardless, breastfeeding provides undoubted health benefits and international expert panels recommend exclusive breastfeeding to 6 months in all infants. Solid complementary foods should not be introduced to infants before 4–6 months of age. There is no evidence that delaying solids including highly allergenic foods has a significant protective effect on the development of atopic disease (Greer *et al*., 2008).

6.2.4 Diagnosis of food allergies

An accurate diagnosis is crucial to providing a food-allergic individual with proper medical care and advice. A list of procedures considered for the diagnosis of IgE-mediated food allergies is given in Box 6.3. The gold standard

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Box 6.3. Diagnostic procedures in food allergies

- Double-blind, placebo-controlled oral food challenge (DBPCFC): documents the role of food in elicitation of symptoms
- Skin-prick test (SPT): identifies the presence of allergen-specific IgE antibodies on skin mast cells
- Serum IgE test: identifies the presence of allergen-specific IgE antibodies in blood serum
- Basophil histamine release test: shows ability of allergen to release histamine and other mediators from basophils *in vitro*

diagnostic method for food allergies is a double-blind placebo-controlled food challenge (DBPCFC) (Taylor & Hefle, 2006; van Ree *et al*., 2006; Boyce *et al*., 2010). DBPCFC is the best method for linking the symptoms to ingestion of a specific food. However, in patients with a history of severe reactions to a particular food, DBPCFCs are often avoided due to the risks involved. Additionally, DBPCFCs are expensive and time-consuming to perform, which leads some clinicians to utilize alternative types of oral challenges (i.e., single-blind or open challenges). These types of challenges can be diagnostic if the challenge is negative or if objective symptoms during the challenge agree with symptoms in the patient's medical history and the allergy is supported by laboratory tests (Boyce *et al*., 2010). Furthermore, with milk allergy in young infants, open challenges are often a preferred approach because the importance of blinding of challenges is less with infants.

DBPCFC and other oral challenges do not provide information on the mechanism of the adverse reaction. However, the immediate onset of an adverse reaction on challenge is often a good indicator of an IgE-mediated food allergy. Other diagnostic methods are used to provide evidence of an IgE-mediated mechanism for the adverse reaction. Skin prick tests (SPTs) are the most common diagnostic tool for confirmation of IgE-mediated food allergies. SPTs have good negative predictive accuracy and are therefore useful for ruling out IgE-mediated food allergies. On the other hand, the positive predictive value of SPTs is not as accurate, and false positives are possible (Sampson & Ho, 1997). Sensitization (the development of specific IgE antibodies) without clinical reactivity to the food can often result in a positive SPT. In general, SPTs are useful for identifying foods potentially responsible for a suspected food allergy, but alone they are not diagnostic for food allergy (Boyce *et al*., 2010).

In addition to the *in vivo* SPT method, *in vitro* methods are also often used for allergenicity assessment. The measurement of allergen-specific IgE in blood serum can be accomplished with several methods, including the radioallergosorbent test (RAST) or the commercial ImmunoCAP system (Pharmacia Diagnostics) (Taylor & Hefle, 2006; van Ree *et al*., 2006). Once again, these methods generally have good negative predictive values but low specificity and poor predictive accuracies when only the presence or absence of antigen-specific IgE antibodies is evaluated (Sampson & Ho, 1997; van Ree *et al*., 2006). As with SPT, screening serum for allergen-specific IgE can help to identify foods potentially responsible for allergic reactions, but does not necessarily give an indication of clinical relevance (Boyce *et al*., 2010). However, when quantitative IgE values are used in conjunction with negative and positive cut-off values, the methods can give better indications of clinical significance (Sampson & Ho, 1997).

Another *in vitro* method for allergenicity assessment is the basophil histamine release test. As mentioned previously, an allergic reaction occurs after an allergenic protein cross-links IgE antibodies present on the surface of effector cells, which then go on to release mediators (Taylor & Hefle, 2006; van Ree *et al*., 2006). IgE cross-linking can be monitored *in vitro* with basophils and potentially allergenic proteins by measuring the release of histamine (or other mediators). Basophil assays have better specificity and correlation to clinical reactivity since they simulate the cross-linking event required for a reaction to occur (van Ree *et al*., 2006).

6.2.5 Prevention and treatment of food allergy

While clinical researchers are actively pursuing treatment methods for food allergies, the only definitive strategy for preventing allergic reactions is avoidance of the causative food. Implementation of an avoidance diet remains the safest practice for food-allergic individuals (Boyce *et al*., 2010). However, avoidance diets are not free from error. If a reaction does occur after accidental ingestion of the allergenic food, pharmacological treatment can help mitigate some of the reaction symptoms. In the case of acute life-threatening reactions, intramuscular epinephrine (e.g., EpiPen™) should be administered rapidly. Delaying epinephrine during anaphylaxis leads to an increased risk of death and morbidity. Milder reactions can be treated successfully with antihistamines (Boyce *et al*., 2010).

A number of research groups have been working on different food allergy treatment methods that aim to prevent allergic reactions, including those to milk. Two studies in particular examined the effectiveness of oral immunotherapy (OIT) in patients with or without a history of anaphylaxis. The first study (Skripak *et al*., 2007) included 23 patients with cows' milk allergy (CMA) without a history of anaphylactic reactions. After a preliminary food challenge to confirm the existence of milk allergy and establish a minimal eliciting dose for each individual, those in the active treatment group were given small (below the minimal eliciting

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dose) increasing amounts of nonfat dry milk over the course of several months. Before treatment, almost all patients had reactions to 40 mg of milk protein. After treatment, reactions in the active group occurred at an average of 5140 mg of milk protein, while the placebo group showed no change in the average reaction dose. Even though the individual thresholds in the active group were increased after treatment, 14 of 18 individuals still had reactions. Researchers concluded that OIT could provide some protection against reactions due to accidental exposure. However, the authors did not have adequate evidence to determine whether the increased thresholds were due to desensitization or the development of oral tolerance. Desensitization indicates a short-term loss or reduction in reactivity to the allergenic food. With desensitization, continued exposure is required to maintain the diminished state of reactivity. Tolerance, on the other hand, is a long-term loss of reactivity that does not require continued maintenance. Differentiation between these two outcomes would require removal of the allergenic food from a patient's diet for a particular period of time before another challenge. Such clinical trials have not yet been performed.

In a study from a different research group (Longo *et al*., 2008), 60 children with severe CMA and at least one severe reaction after accidental milk exposure were enrolled in an OIT program. The therapy was organized into two phases, the first of which (the rush phase) was performed in a hospital. Subjects in the active group were given rapidly increasing amounts of milk over the course of 10 days, while on a constant dose of antihistamines. After completing the rush phase, the subjects entered the home phase where they were given gradually increasing levels of milk. Antihistamines continued to be administered at home. After 1 year, 36% of the active group could tolerate more than 150 mL of milk, 54% could tolerate 5–150 mL, and 10% did not continue the program due to development of symptoms during OIT. The placebo group experienced no change in the minimal reactive dose. Once again, researchers concluded that the OIT resulted in partial desensitization, which could provide some protection against accidental exposure.

These studies and others like them are a significant step toward development of an effective milk allergy treatment. However, OIT remains an experimental treatment and further studies will be needed to document the safety and efficacy of this approach. For the majority of individuals with CMA, the only definite way to prevent reactions is to completely remove milk from their diets.

The food-allergic consumer is faced with the responsibility to construct and adhere to safe and effective avoidance diets. Avoidance diets are typically constructed under the admonition to entirely avoid the specific allergenic food(s) and all ingredients derived from that food (Taylor *et al*., 1999). Food-allergic consumers often have questions about the source of ingredients and their degree of allergenic risk associated with specific food ingredients. Labeling terms (e.g., casein) that do not clearly disclose the source of the ingredient are also confusing. In the USA, the passage of the Food Allergen Labeling and Consumer Protection Act in 2004 mandated the use of plain English to divulge the source of ingredients such as casein and the presence of commonly allergenic foods in ingredients that could otherwise be declared by collective terms such as natural flavor. Some foods, such as restaurant foods, are not labeled in most countries and can present an increased level of allergic risk as a consequence.

While food-allergic consumers are advised to avoid all sources of the specific allergenic food, small (sometimes extremely small) doses of the allergenic food can be safe (Taylor *et al*., 2002). Low milligram levels, and even sometimes sub-milligram levels, are demonstrably safe for food-allergic individuals in clinical oral challenge trials (Taylor *et al*., 2010). Thus, the allergenic load of ingredients derived from an allergenic food is an important determinant of the degree of allergenic risk. For example, casein or whey protein concentrate have high allergenic risk because they contain large amounts of allergenic protein, while lactose has a lower risk because it contains a much lower level of residual milk protein. However, scientific and regulatory consensus has not yet been achieved with respect to acceptable threshold levels for allergenic foods so labeling continues to reflect the reality of attempts to achieve complete avoidance.

6.2.6 Cows' milk and avoidance diets

Cows' milk is a particularly important food in the diets of infants. Cows' milk supplies a significant amount of calories, fat and protein as well as vitamins and minerals including calcium, vitamin D, and B vitamins (United States Department of Agriculture, 2010). Dietary interventions and avoidance strategies must be continually reevaluated as the child grows and transitions from a liquid diet of breast milk and/or formula to a mixed diet of solids and liquids, as well as the possibility of CMA remission. Noncompliance with recommendations can lead to inappropriate diets, hyperrestrictions, and malnutrition (Fiocchi *et al*., 2010a). Therefore, an individualized dietary regimen adequate in nutrition must be considered for infants and children affected by CMA. Weaning from a formula to a milk substitute (fortified rice or soy milk) will vary depending on the current diet and individual needs assessment. Generally, milk substitutes may be introduced after 1 year of age and when the diet (calories) comes from a mixed diet of solids and liquids and the child shows good growth velocity (Venter, 2009).

Individuals with CMA must avoid ingesting the causative food(s) to prevent an allergic reaction. In exclusively

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breast-fed infants, this requires strict maternal elimination of the cows' milk protein from the diet. Human milk contains intact food allergens, including traces of cows' milk proteins from the mother's diet (Lifschitz *et al*., 1988). In formula-fed children, an extensively hydrolyzed protein formula (eHF) is often the first choice for those with documented CMA as residual milk protein peptides in partially hydrolyzed formulas may cause an allergic reaction (Greer *et al*., 2008). In extremely sensitive individuals, amino acid formulas may be required (Høst & Halken, 2004). Amino acid formulas are made up of free amino acids and are considered nonallergenic. Amino acid formulas are considerably more expensive and have a more distinct flavor than eHFs so this may need to be considered when recommending their use (Hill *et al*., 2007).

6.3 DELAYED FOOD ALLERGIES

In addition to IgE-mediated reactions, some types of food allergy are classified as cell-mediated allergies. These types of disorders are also often referred to as delayed-type hypersensitivity, as the symptoms occur 6–24 hours after the offending food has been ingested (Taylor & Hefle, 2006). The symptoms of cell-mediated food allergies arise when food proteins stimulate specific T cells, which go on to produce inflammatory mediators including certain cytokines and chemokines. The inflammatory responses resulting from mediator release are generally isolated to the gastrointestinal tract, where a majority of the symptoms occur (Taylor & Hefle, 2006). Primary examples of cell-mediated food allergies include celiac disease and food protein-induced enterocolitis syndrome, the latter of which is discussed in section 6.7 (Sampson, 2004). Lastly, eosinophilic gastrointestinal disorders (including allergic eosinophilic esophagitis and gastroenteritis) are the result of IgE-mediated and/or cell-mediated mechanisms (Sampson, 2004).

6.4 COWS' MILK ALLERGY

CMA is the most common IgE-mediated food allergy in infants and children, occurring at a rate of 2–3% in this population (Høst & Halken, 1990; Wal, 2004; Monaci *et al*., 2006; Skripak *et al*., 2007). Many of the children affected by CMA will eventually outgrow the disease. However, the frequency of this phenomenon is not well defined. Original estimates, based on a study of Danish infants with CMA (Høst & Halken, 1990), indicated a recovery rate of 87% by age 3 years. A more recent study in the USA (Skripak *et al*., 2007) indicated that only 5–19% of children outgrew the allergy by age 4 years and 55–79% outgrew CMA by age 16. While the researchers in this study found several factors that could be useful in predicting the course of the allergy, the development of tolerance should be assessed on an individual basis.

As with other food allergies, CMA symptoms can range from mild to life-threatening, and reactions to milk can involve gastrointestinal, cutaneous, respiratory, and generalized symptoms (Taylor & Hefle, 2006). In one study of children with CMA, 64% of patients reported cutaneous symptoms, 59% experienced gastrointestinal symptoms, 33% reported respiratory symptoms, and 36% experienced symptoms in more than one of these categories (Skripak *et al*., 2007). The literature also contains several reports of anaphylaxis in patients with CMA who accidentally consumed milk (Collins-Williams, 1955, 1956; Sampson *et al*., 1992; Tarim *et al*., 1994; Laoprasert *et al*., 1998).

CMA can often present with symptoms similar to those of lactose intolerance, including diarrhea and stomach cramps. However, lactose intolerance will not induce hives or respiratory problems. Another important diagnostic difference is that onset of a CMA reaction is immediate, while a lactose intolerance reaction is delayed by several hours (Monaci *et al*., 2006). The differences in the symptoms of these two conditions are due to their reaction induction mechanisms. Lactose intolerance, which is not mediated by the immune system, is due to a deficiency of β-galactosidase. In the absence of this enzyme in the digestive tract, lactose is not hydrolyzed into its component monosaccharides but instead passes into the colon where bacteria metabolize the sugar. The products of this fermentation (CO_2, H_2, and H_2O) are responsible for the gastrointestinal distress associated with lactose intolerance (Taylor & Hefle, 2006).

Proteins are the most important components of milk in terms of allergies. Typical bovine milk contains 3.0–3.5% protein, and milk proteins can be divided into two categories based on their solubility at pH 4.6 (Swaisgood, 2003; Monaci *et al*., 2006). Whey proteins, which are soluble at pH 4.6, account for approximately 20% of the total milk protein fraction. Caseins represent the remaining 80% of milk proteins and are insoluble at pH 4.6 (Monaci *et al*., 2006; Fox, 2009).

Both of these fractions contain numerous individual proteins, many of which have been shown to be capable of sensitizing susceptible individuals and eliciting allergic reactions (Wal, 2004). However, the major allergens are whole casein (CN) and the whey proteins β-lactoglobulin (BLG) and α-lactalbumin (ALA) (Wal, 2004). Major allergens are considered to be those proteins against which at least 50% of allergic individuals have specific IgE (Taylor & Hefle, 2006). One study found that 65% of milk-allergic individuals were sensitized to CN, 61% to BLG, 51% to ALA, 43% to bovine serum albumin (BSA), 36% to immunoglobulins, and 35% to lactoferrin (Wal, 2002). However, it is important to note that sensitization (presence of specific IgE) does not alone demonstrate that the protein is an allergen. Thus, the analysis indicated that only 26% of individuals were monosensitized, indicating that most patients

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with CMA have antibodies recognizing more than one milk protein (Wal, 2002), although perhaps one was more important than another. CN, BLG, and ALA are the milk proteins that are identified unequivocally as allergens. As the whey and casein fractions of milk proteins are complex mixtures of individual proteins, an examination of the individual proteins is essential in order to understand CMA.

6.4.1 Whey proteins

6.4.1.1 β-*Lactoglobulin*

BLG is the most abundant whey protein in cow milk, accounting for 50% of the whey fraction and 10% of the total protein (Edwards *et al*., 2009; Fox, 2009). BLG is found in milk from ruminants and some monogastrics but not in the milk of humans, camels, rodents, or lagomorphs (Sawyer, 2003; Edwards *et al*., 2009; Sheehy *et al*., 2009). As a member of the lipocalin protein family, BLG has structural features that allow it to bind hydrophobic molecules in milk including retinol and fatty acids (Edwards *et al*., 2009; Fox, 2009; Sheehy *et al*., 2009).

The structure of BLG has been studied quite extensively. The protein has a molecular mass of 18.3 kDa and is composed of 162 amino acids (Edwards *et al*., 2009; Fox, 2009; Sheehy *et al*., 2009). The first correct primary sequence was identified by Braunitzer *et al*. in 1972 (Sawyer, 2003). Since then researchers have identified 10 genetic variants, with two (A and B) being the most abundant (Sawyer, 2003; Edwards *et al*., 2009; Sheehy *et al*., 2009). The sequence for variant B is displayed here (UniProt Knowledgebase LACB_BOVIN, 2002–2010):

LIVTQTMKG LDIQKVAGTW YSLAMAASDI SLLDAQSAPL RVYVEELKPT
PEGDLEILLQ KWENGECAQK KIIAEKTKIP AVFKIDALNE NKVLVLDTDY
KKYLLFCMEN SAEPEQSLAC QCLVRTPEVD DEALEKFDKA LKALPMHIRL
SFNPTQLEEQ CHI

In terms of secondary structure, BLG is composed of 10–15% α-helix, 43% β-sheet, and 47% random coil (Edwards *et al*., 2009). The tertiary structure is similar to that of plasma retinol-binding protein, having an eight-strand β-barrel flanked by a three-turn α-helix (Sawyer, 2003; Edwards *et al*., 2009). Two disulfide bonds are present from Cys66 to Cys160 and Cys106 to Cys119 (Edwards *et al*., 2009). In addition, there is a free thiol group at Cys121, which is responsible for the formation of disulfide-linked dimers at physiological pH (5.5–7.5) (Edwards *et al*., 2009; Fox, 2009). At lower pH (<3.5), BLG is in a monomeric form, and at pH above 7.5 BLG exists as a tetramer (Fox, 2009).

BLG is one of the major allergens in cows' milk, with a reported sensitization rate of 61% (Wal, 2002; Monaci *et al*., 2006). In addition, other studies have reported that 90% of patients had IgE that recognized BLG peptides (Wal, 2004). A number of researchers have investigated the linear epitopes recognized by IgE from patients with CMA, using either BLG digested with trypsin or synthetic BLG peptides (Ball *et al*., 1994; Heinzmann *et al*., 1999; Sélo *et al*., 1999; Järvinen *et al*., 2001). When these studies are taken together, however, the identified epitopes cover almost the entire length of BLG's primary sequence.

BLG has a number of physiochemical properties that are important to both its allergenicity and its stability in food systems. BLG is extremely resistant to digestion, and has even been found at low levels in fecal material (Wal, 2004; Monaci *et al*., 2006). The tremendous digestive stability likely plays a prominent role in BLG's ability to sensitize individuals and elicit reactions (Wal, 2002). In contrast to its proteolytic stability, BLG is quite susceptible to unfolding on thermal treatment. According to differential scanning calorimetry, the midpoint of unfolding is 75 °C and BLG is completely unfolded after 10 min at 90 °C (Edwards *et al*., 2009; Fox, 2009).

6.4.1.2 α-*Lactalbumin*

The second most abundant whey protein is ALA, accounting for approximately 20% of the whey protein fraction (Fox, 2009). Composed of 123 amino acids, ALA has a molecular mass of approximately 14.2 kDa (Brew, 2003; Edwards *et al*., 2009; Fox, 2009; Sheehy *et al*., 2009). ALA is present in the milk of all mammals and exhibits significant interspecies homology (Brew, 2003; Sheehy *et al*., 2009). In bovine breeds, two genetic variants are possible, and of the two B is the more common (Sheehy *et al*., 2009). ALA plays an essential role in lactose synthesis by regulating the lactose synthase enzyme complex (Brew, 2003; Edwards *et al*., 2009; Fox, 2009). The amino acid sequence for variant B of ALA is shown here (UniProt Knowledgebase LALBA_BOVIN, 2002–2010):

EQLTKCEVF RELKDLKGYG GVSLPEWVCT TFHTSGYDTQ AIVQNNDSTE
YGLFQINNKI WCKDDQNPHS SNICNISCDK FLDDDLTDDI MCVKKILDKV
GINYWLAHKA LCSEKLDQWL CEKL

ALA has a substantial amount of structural homology with lysozyme proteins including those from chickens and humans (Brew, 2003; Edwards *et al*., 2009). The tertiary structure of ALA is principally defined by the presence of two lobes, α and β (Brew, 2003; Edwards *et al*., 2009). The α lobe consists of three α-helices while the β lobe is composed of a small three-strand β-pleated sheet (Brew, 2003). The two lobes share a disulfide bond between Cys73 of the β lobe and Cys90 of the α lobe. In addition there are three other disulfide bonds: Cys6–Cys120 and Cys28–Cys111 in the α lobe and Cys60–Cys77 in the β lobe (Brew, 2003).

Unlike lysozymes, however, ALA binds calcium quite tightly (Brew, 2003). The elbow between the two lobes of ALA serves as a binding site for calcium ions (Brew, 2003;

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Edwards *et al*., 2009). The binding of Ca^{2+} affects the thermal stability of ALA. While holo-ALA unfolds at a lower temperature than BLG, the apo form has an even lower denaturation temperature (Edwards *et al*., 2009). In addition, ALA has the ability to refold after denaturation, and the presence of calcium ions accelerates the refolding process (Edwards *et al*., 2009).

ALA is considered to be one of the major cows' milk allergens, with one study reporting that 51% of individuals tested had IgE antibodies specific for ALA (Wal, 2002; Monaci *et al*., 2006). As was the case with BLG, researchers have investigated the specificity of CMA-patient IgE for specific peptides produced synthetically or from tryptic digestion (Maynard *et al*., 1997; Järvinen *et al*., 2001). The peptides identified by two research groups cover nearly the entire length of ALA's primary sequence.

Unlike BLG, ALA is relatively susceptible to digestion. Authors examining the digestive stability of purified ALA in simulated gastric fluid have reported incubation times of 30 s to 5 min as being sufficient to fully hydrolyze the protein, while incubation in patient gastric secretions required 30 min for complete hydrolysis (Jakobsson *et al*., 1982; Fu *et al*., 2002; Moreno *et al*., 2005). However, the stability of ALA is dramatically enhanced when the simulated digestion is performed with milk rather than with the purified protein (Jakobsson *et al*., 1982). Moreno *et al*. (2005) investigated milk phosphatidylcholine (PC) as one possible stabilizing factor. When ALA underwent simulated digestion in the presence of PC, the protein was still detected after 60 min while the ALA digested without PC was not detected in incubations lasting more than 5 min. These results emphasize the complex interactions between food allergens and food matrices.

6.4.1.3 Minor whey proteins

In addition to BLG and ALA, the whey fraction of milk contains a number of other proteins. BSA is present at approximately 0.1–0.4 g/L (0.001–0.004%) in whole milk (Fox 2009). Bovine immunoglobulins are found at levels between 0.06 and 0.1% in whole milk. Since no immunoglobulins are transferred *in utero*, the presence of these proteins in milk and at high levels in colostrum (10%) serve to provide initial immunity to the calf (Fox, 2009). Lactoferrin is an iron-binding protein present at low levels in milk, accounting for less than 1% of whey protein (Wal, 2004; Monaci *et al*., 2006). Lactoferrin is known for its bacteriostatic properties (Edwards *et al*., 2009). The clinical relevance of these minor milk proteins is probably negligible as they are present at very low levels, and individuals with CMA with IgE antibodies specific for these proteins generally have antibodies against other and more prominent milk proteins as well (Monaci *et al*., 2006).

6.4.2 Caseins

The casein fraction of milk protein traditionally includes those proteins that precipitate at pH 4.6 (Swaisgood, 2003; Fox, 2009). While commonly mistaken for one protein, the casein fraction actually consists of several proteins. Casein proteins can be classified as either calcium-sensitive or calcium-insensitive depending on whether they form aggregates in the presence of calcium ions (Fox, 2009). Calcium-sensitive caseins have clusters of phosphorylated serine residues, resulting in electrostatic repulsion between the proteins and preventing aggregate formation (Swaisgood, 2003; Holland, 2009; Horne, 2009). The presence of calcium ions neutralizes electrostatic repulsions between phosphoserine clusters, which causes the caseins to aggregate (DeKruif & Holt, 2003; Swaisgood, 2003; Horne, 2009). In bovine milk, calcium-sensitive caseins include α_{s1}-, α_{s2}-, and β-casein.

Representing approximately 40% of the casein fraction in cow milk, α_{s1}-casein is a 23.6-kDa protein composed of 199 amino acid residues (Monaci *et al*., 2006; Fox, 2009; Sheehy *et al*., 2009). Eight genetic variants have been characterized, with variant B being the most common in *B. taurus* (Sheehy *et al*., 2009). The primary sequence for α_{s1}-casein (variant B) is displayed with the phosphorylated residues underlined (UniProt Knowledgebase CASA1_BOVIN, 2002–2010):

RPKHPIKHQ GLPQEVLNEN LLRFFVAPFP EVFGKEKVNE LSKDIGSEST
EDQAMEDIKQ MEAESISSSE EIVPNSVEQK HIQKEDVPSE RYLGYLEQLL
RLKKYKVPQL EIVPNSAEER LHSMKEGIHA QQKEPMIGVN QELAYFYPEL
FRQFYQLDAY PSGAWYYVPL GTQYTDAPSF SDIPNPIGSE NSEKTTMPLW

The next casein, α_{s2}-casein, has a molecular mass of 25.2 kDa and accounts for 12.5% of the casein fraction in cow milk (Monaci *et al*., 2006; Sheehy *et al*., 2009). The primary sequence of 201 amino acid residues is shown with the phosphorylated residues again underlined (Holland, 2009; UniProt Knowledgebase CASA2_BOVIN, 2002–2010):

KNTMEHVSS SEESIISQET YKQEKNMAIN PSKENLCSTF CKEVVRNANE
EEYSIGSSSE ESAEVATEEV KITVDDKHYQ KALNEINQFY QKFPQYLQYL
YQGPIVLNPW DQVKRNAVPI TPTLNREQLS TSEENSKKTV DMESTEVFTK
KTKLTEEEKN RLNFLKKISQ RYQKFALPQY LKTVYQHQKA MKPWIQPKTK
VIPYVRYL

The second most abundant casein is β-casein, representing 35% of whole casein in cow milk (Monaci *et al*., 2006; Fox, 2009). This 24-kDa protein is composed of 209 amino acids and exists as one of 12 genetic variants (Sheehy *et al*., 2009). The A2 variant is shown here with its phosphorylation sites underlined (Holland, 2009; UniProt Knowledgebase CASB_BOVIN, 2002–2010):

RELEELNVP GEIVESLSSS EESITRINKK IEKFQSEEQQ QTEDELQDKI
HPFAQTQSLV YPFPGPIPNS LPQNIPPLTQ TPVVVPPFLQ PEVMGVSKVK
EAMAPKHKEM PFPKYPVEPF TESQSLTLTD VENLHLPLPL LQSWMHQPHQ
PLPPTVMFPP QSVLSLSQSK VLPVPQKAVP YPQRDMPIQA FLLYQEPVLG
PVRGPFPIIV

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In contrast to the calcium-sensitive caseins described above, calcium-insensitive proteins are not phosphorylated and are therefore not precipitated by calcium ions. In bovine milk, the only calcium-insensitive casein is κ-casein (Fox, 2009). κ-Casein is present in whole casein at 12.5% (Monaci *et al*., 2006; Fox, 2009). This protein has a molecular mass of 19 kDa and is composed of 169 amino acids, the sequence of which is shown here (Sheehy *et al*., 2009, UniProt Knowledgebase CASK_BOVIN, 2002–2010):

QEQNQEQPI RCEKDERFFS DKIAKYIPIQ YVLSRYPSYG LNYYQQKPVA
LINNQFLPYP YYAKPAAVRS PAQILQWQVL SNTVPAKSCQ AQPTTMARHP
HPHLSFMAIP PKKNQDKTEI PTINTIASGE PTSTPTTEAV ESTVATLEDS
PEVIESPPEI NTVQVTSTAV

Unlike the other caseins, κ-casein has no phosphoserine clusters (Swaisgood, 2003; Holland, 2009). Instead, κ-casein is glycosylated at up to six threonine residues, as indicated by the underlining in the above sequence (Holland, 2009; Sheehy *et al*., 2009). Even with the lack of phosphorylation, κ-casein still has distinct polar and nonpolar regions. The N-terminal portion of the protein (amino acid residues 1–105) is quite hydrophobic, while the C-terminal region is polar. Also unlike the other caseins, κ-casein exists as a monomer or as disulfide-linked oligomers composed of up to eight or more monomer units (Swaisgood, 2003; Holland, 2009).

Caseins have a set of distinctly unique characteristics due to their lack of secondary structures. Caseins are neither globular nor fibril nor random but are instead rheomorphic proteins having open structures and high degrees of side-chain and backbone flexibility (DeKruif & Holt, 2003; Fox, 2009). Unlike whey proteins, caseins in milk exist in superstructures known as micelles (Fox, 2009). Casein micelles are typically 50–500 nm in diameter with molecular masses between 10^6 and 10^9 Da (Horne, 2009). The presence of micelles causes the hallmark white color of milk due to their light-scattering ability (Fox, 2009; Horne, 2009).

Several models including the submicelle, nanocluster, and dual-binding models have been proposed (Walstra, 1990; DeKruif & Holt, 2003; Horne, 2009). While there are differences among the models, the general picture of a casein micelle remains similar. As mentioned previously, the association of caseins via interactions in their hydrophobic regions is countered by the electrostatic repulsion of the phosphoserine clusters. In some aqueous systems, this balance keeps caseins from precipitating. Milk, however, has the very important characteristic of being supersaturated with calcium phosphate (Swaisgood, 2003; Fox, 2009). Despite the presence of high levels of calcium ions, caseins do not spontaneously precipitate in cows' milk because the presence of κ-casein stabilizes the calcium-sensitive caseins. The bulk of the micelle interior is composed of α_{s1}-, α_{s2}-, and β-caseins aggregated with Ca^{2+} while κ-casein forms a "hairy layer" on the surface of micelles with its C-terminus providing a layer of stearic hindrance that prevents micelles from aggregating (DeKruif & Holt, 2003).

The structural characteristics of individual caseins and micelles play an important role in the digestive and thermal stabilities of these allergens. Enzymatic proteolysis can play an important role in micelle solubility. For example, in the cheesemaking process chymosin hydrolyzes κ-casein, and without protective layers micelles precipitate in the presence of calcium ions (Horne, 2009). In terms of human gastrointestinal digestion, the open structure of whole casein makes it especially susceptible to many types of proteases (Fox, 2009). On the other hand, the rheomorphic nature of caseins yields a high degree of thermal stability (DeKruif & Holt, 2003).

The allergenicity of the caseins is commonly assessed based on IgE binding to whole casein. Between 63 and 65% of sera from patients with CMA have been shown to contain casein-reactive IgE antibodies (Bernard *et al*., 1998; Wal, 2002). However, researchers have also investigated the prevalence of IgE against each of the individual casein proteins (Bernard *et al*., 1998). The analysis indicated that of the 58 casein-sensitized patient sera, 57 showed reactivity against α_{s1}-casein, 55 against α_{s2}-casein, 53 against β-casein, and 58 against κ-casein.

Researchers have also examined the linear epitopes present on each of the individual casein proteins. Several research groups have mapped the IgE-binding epitopes of α_{s1}-casein using synthetic decapeptides, synthetic 20-mer peptides, or cyanogen bromide digests of α_{s1}-casein (Spuergin *et al*., 1996; Nakajima-Adachi *et al*., 1998; Chatchatee *et al*., 2001a). As noted with other milk proteins, the combined results of these studies appear to indicate that sequential IgE epitopes cover the entire length of the protein.

Interestingly, the study by Chatchatee *et al*. (2001a) identified two linear epitopes that were recognized only by the antibodies from older patients with persistent CMA and not by antibodies from patients that would go on to outgrow their allergy. The idea that the two manifestations of CMA (persistent and transient) are the result of differential epitope recognition is also used by this research group to explain varying reactions to thermally processed milk, as discussed in section 6.6.

The IgE-binding patterns of α_{s2}-casein have been studied somewhat less than the other milk proteins discussed thus far. Two major and two minor IgE-binding epitopes were identified using synthetic decapeptides (Busse *et al*., 2002). Epitope mapping of β-casein also revealed several major and minor epitopes (Chatchatee *et al*., 2001b). Similar to

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observations with α_{s1}-casein, two of the minor β-casein epitopes were only recognized by IgE from older CMA patients. In the case of κ-casein, the major epitopes identified by the analysis covered almost the entire primary sequence of the protein. Young patients, however, only recognized two regions: amino acid residues 21–44 and 53–64 (Chatchatee *et al*., 2001b).

6.5 CROSS-REACTIVITY WITH MILK FROM OTHER SPECIES

In addition to cows' milk, a number of other mammalian milks (e.g., goat and sheep) are also used to produce commercial food products. While these nonbovine milks were once suggested as an alternative for individuals with CMA, the use of goat and sheep milk has since been shown to be unsafe for individuals with CMA. Several studies have shown extensive *in vitro* IgE cross-reactivity among cow, sheep, and goat milk proteins (Spuergin *et al*., 1997; Restani *et al*., 1999). In addition, a DBPCFC study indicated that 24 of 26 patients with CMA reacted to goat milk (Bellioni-Businco *et al*., 1999). The reactions to nonbovine milk can be severe as seen in a case of anaphylaxis following goats' milk ingestion by an infant with previously documented CMA (Pessler & Nejat, 2004). Because of the strong possibility of cross-reactivity, sheep and goat milk products are not recommended for individuals with CMA. Interestingly, in a study of reindeer milk, also a ruminant, only partial cross-reactivity of human anti-bovine IgE with reindeer BLG was shown in sera from 21 CMA children (Suutari *et al*., 2006).

In rare cases, goat and/or sheep milk allergy can be seen in the absence of reactivity to cows' milk (Calvani & Alessandri, 1998; Umpiérrez *et al*., 1999; Ah-Leung *et al*., 2006). The characterization of 28 patients with goat and sheep milk (GSM) allergy revealed key differences from CMA. The onset of GSM allergy generally occurred at a later age than CMA, with a mean age of 6 years at the first reaction. The IgE present in the sera of these allergic individuals reacted with the calcium-sensitive GSM caseins (α_{s1}-, α_{s2}-, and β-caseins), but showed little or no binding to κ-casein or whey proteins (including BLG). In addition, the researchers observed no cross-reactivity with bovine proteins (Ah-Leung *et al*., 2006). Clearly, isolated GSM allergy is a condition distinct from CMA.

Cross-reactivity between milks from different mammalian species has been reported and depends somewhat on phylogenentic relationship (Restani *et al*., 1999, 2009). Highest homologies are observed between milk proteins of cow (Bovidae), buffalo (96.1%), sheep (91.1%), and goat (87.6%). Using milks more phylogenetically distant from Bovidae has better clinical results (Järvinen & Chatchatee, 2009).

Buffalo milk shows nearly 96.1% homology to cows' milk (Restani *et al*., 1999, 2009). Twenty four CMA patients studied had positive SPTs to deer, ibex and buffalo milk while 25% tested positive to camel milk and 20% to pig milk (Katz *et al*., 2008). Anaphylaxis has also been reported to mozzarella cheese from buffalo milk in a non-CMA adult (Broekaert *et al*., 2008).

Horse and donkey milks (equine) have also been suggested as an alternative dietary option in CMA. Nine infants with multiple food allergies, including CMA, were successfully treated with ass' milk over several months (Iacono *et al*., 1991). In a randomized crossover study, donkey milk was better tolerated in CMA children with atopic dermatitis. In DBPCFC of 26 children, 24 had a positive reaction with goat milk and one with donkey milk (Vita *et al*., 2007). Businco *et al*. (2000) showed that mares' milk was tolerated in 24 of 25 children with severe CMA, although one child reacted on DBPCFC to mares' milk (2000). There are at least two reports of anaphylaxis to mares' milk in adults without CMA (Gall *et al*., 1996; Fanta & Ebner, 1998).

Camel (dromedary) milk has a high proportion of β-casein and absence of BLG, similar to human milk. In *in vitro* studies in which milks of different mammalian species are incubated with monoclonal or polyclonal antibodies produced against cows' milk proteins, none of the six CMA children showed a positive response to camel milk proteins (Restani *et al*., 1999). In a similar study, Western blot analysis resulted in the absence of immunological cross-reactivity between camel and cow milk proteins (El-Agamy *et al*., 2009). Homology with cows' milk proteins are only 60%, the lowest level of similarity. In a prospective cohort study, Ehlayel *et al*. (2011) performed SPTs to camel milk on 35 CMA children. All 28 patients with negative SPTs tolerated camel milk on ingestion without any reactions. However, the seven children with positive SPTs were not orally challenged to camel milk.

There is at least one report of near-fatal anaphylaxis to camel milk in a young boy with history of asthma, eczema, and multiple food allergies but negative for CMA (Al-Hammadi *et al*., 2010). It should also be noted that camel milk is not a kosher food product and would not be acceptable to kosher dietary laws. When using any mammalian milk substitute, a supervised oral challenge is strongly recommended as there is no hypoallergenic mammalian milk. Cross-reactivity is frequently encountered with milk from other species. Some individuals with CMA are able to tolerate the milk of other species but this is not consistently observed in all CMA subjects. Furthermore, unique allergies to milk from other species in individuals tolerant of cows' milk is also occasionally observed.

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6.6 EFFECTS OF PROCESSING ON ALLERGENICITY

Food products are processed for a number of reasons: to improve safety, to improve stability, to enhance organoleptic features, and to provide specific functional properties. A wide variety of processes is used in food manufacturing including milling, heating, chilling, and fermentation (Poms & Anklam, 2004). Theoretically, any process that changes the proteins in an allergenic food has the potential to change the allergenicity and/or immunochemical detection of the food proteins.

Thermal processing is particularly common in the food industry, and many of these processes have the potential to alter the detection and allergenicity of food allergens. The application of heat to a food system has the potential to induce changes in protein conformation. Since IgE antibodies recognize specific areas on a protein, any process that alters the protein can also alter the binding capacity of the antibodies. IgE epitopes can broadly be classified as linear or conformational. Linear epitopes consist of short peptide fragments and recognition is based on the amino acid sequence of the peptide. Conformational epitopes, however, are those that involve the three-dimensional structure of the protein (Poms & Anklam, 2004). Heating can cause protein unfolding, which could disrupt conformational epitopes and result in decreased IgE binding to those particular areas (Nowak-Wegrzyn & Fiocchi, 2009). However, linear epitopes may still be present and heating can even expose new or previously inaccessible epitopes, increasing the IgE-binding capacity (Besler *et al*., 2001; Mills & Mackie, 2008; Nowak-Wegrzyn & Fiocchi, 2009).

Before reaching the consumer, milk and products derived from milk typically undergo some type of thermal processing. Under Pasteurized Milk Ordinances, Grade A fluid milk must undergo pasteurization (United States Food and Drug Administration, 2007). Commonly used pasteurization procedures include batch (63°C for 30 min) and high-temperature short-time (HTST; 72°C for 15 s) pasteurization (United States Food and Drug Administration, 2007). Ultra high temperature (UHT) processing can also be implemented to produce shelf-stable milk (Douglas *et al*., 1981). In addition, fluid milk is also used to produce a number of products including nonfat dry milk, which can then be used in a variety of foods.

A number of thermally induced interactions among milk proteins have been described in the literature. One process of interest is the denaturation and aggregation of BLG, which involves a multistep reaction including thiol interchange when the protein is exposed to temperatures above 70 °C (DeWit & Klarenbeek, 1984; Griffin *et al*., 1993; Iametti *et al*., 1996). Heating can also induce interactions between different milk proteins, including BLG and κ-casein. When milk is heated, these two proteins form complexes stabilized by disulfide bonds (Euber & Brunner, 1982). Because of the rheomorphic nature of caseins, they do not exhibit the types of thermal denaturation seen with globular proteins (DeKruif & Holt, 2003). Casein micelles will form aggregates on exposure to substantial amounts of heat, although the heat coagulation time is dependent on pH. When the milk is heated at a pH less than 6.8, BLG and κ-casein form complexes on the surface of the casein micelle, but when the pH is greater than 6.8 the complexes form in the serum (Walstra, 1990; Singh & Creamer, 1991). The presence of the BLG–κ-casein complexes at the surface of the casein micelle causes the micelles to be more resistant to the effects of future aggregating conditions including heat, calcium ions, ethanol, and chymosin (Singh & Creamer, 1991). Casein micelles depleted of κ-casein are less stable to subsequent thermal and chemical treatments (Walstra, 1990; Singh & Creamer, 1991).

Milk proteins can also interact with other components of a food matrix during thermal processing. One of the more significant reactions involving milk proteins is the Maillard browning reaction (Morgan *et al*., 1999). In general terms, Maillard browning is a reaction between an amino group and a reducing sugar (Van Boekel, 1998). In a milk system, the amine is contributed by a free amino group (i.e., a lysine residue) on a milk protein and the reducing sugar is in the form of lactose. After this initial condensation, other reaction cascades can occur, resulting in a number of characteristic products including low-molecular-weight flavor compounds and melanoidin pigments responsible for brown color (Van Boekel, 1998). Maillard reactions in milk products can have a number of negative nutritional effects including loss of nutritive value (by blocking lysine residues), decreased digestibility, and enzyme inhibition (Van Boekel, 1998).

The effects of Maillard reactions on milk protein stability seem to be highly dependent on the conditions under which the system is processed. In addition to factors such as time and temperature, the water activity of the glycation system has an effect on milk protein stability (Morgan *et al*., 1999). When comparing Maillard reactions between BLG and lactose, which took place either in a dry system (65% relative humidity) or in solution, researchers found marked differences in protein conformation and solubility (Morgan *et al*., 1999). Under dry conditions, the native conformation and association of BLG was preserved while the protein became highly glycated. When BLG and lactose were processed in solution, however, the product was highly susceptible to proteolysis and had decreased solubility. Another study (Chevalier *et al*., 2001) also reported the formation of several forms of BLG including polymers

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and aggregates stabilized by disulfide and covalent bonds after heating in solution in the presence of various sugars.

While studies examining thermally induced interactions among milk components are quite prevalent in the literature, few studies have explored the interaction of milk proteins with nonmilk constituents in a processed food matrix. However, research has been published which examined the effects of heating egg white proteins (specifically ovomucoid) with other food matrix proteins (Kato *et al*., 1999). The study found that heating ovomucoid in the presence of wheat gluten decreased the detection of ovomucoid by ELISA. Since the incorporation of 2-mercaptoethanol increased the ovomucoid signal, the researchers concluded that the two proteins formed disulfide linkages, which prevented detection. It seems possible that milk proteins have the potential to participate in similar reactions as many of the thermal modifications discussed in this section were mediated by disulfide bonds.

While the general theory of how processing can affect allergenicity and detection is fairly straightforward, the assessment of such effects is considerably more difficult. Whether working with *in vivo* or *in vitro* techniques, a number of challenges can face researchers attempting to interpret their results and compare the results with those from other studies. First, the precise degree of processing can have a considerable effect on the allergenicity and detection of various food products. Second, an often-overlooked factor in the assessment of allergenicity or detection is the solubility of the proteins before and after processing. Third, a large amount of variability can be encountered between patients (in the case of *in vivo* testing) and assays (in the case of *in vitro* testing). Lastly, the matrix in which the processing takes place can have an impact on the resulting allergenicity or detection. Keeping these four factors in mind, the following paragraphs review several examples of how processing can affect allergenicity and detection.

As mentioned previously, the most conclusive way to determine the allergenicity of a particular food is a DBPCFC (Taylor & Hefle, 2006; van Ree *et al*., 2006). Even when using this gold standard for determining allergenicity, differences can arise due to the use of varying degrees of processing. Thermal processing of milk products is a pertinent example of the importance of the degree of processing. One of the earliest analyses of the effects of heating on milk allergenicity examined differences in the reactivity to raw and processed milks (Høst & Samuelsson, 1988). The researchers performed DBPCFCs with three types of milk: raw milk, pasteurized (75°C for 15 s) milk, and homogenized (60°C and 175 kg/cm^3)/pasteurized milk. While the sample size was quite small (five children), all types of milk were shown to provoke a reaction. The reaction times and thresholds varied, with shorter reaction times observed with the homogenized/pasteurized samples. In this case, the heat treatment did not eliminate or reduce allergenicity.

A more recent study by Nowak-Wegrzyn *et al*. (2008) investigated the allergenicity of milk in a thermally processed food matrix. The researchers challenged milk-allergic patients with two foods: a muffin baked at 177°C (350°F) for 30 min and a waffle cooked at 260°C (500°F) for 3 min. Each of the samples contained a total of 1.3 g of milk protein. Of 100 children challenged with the foods, 68% tolerated the baked products, 23% reacted to the baked products, and 9% were tolerant to both baked and pasteurized milk.

The authors proposed that CMA presents as one of two different phenotypes. The first is transient allergy, in which the patients have antibodies directed primarily against conformational epitopes. The individuals with transient CMA could tolerate the baked milk products because the processing alters those conformational epitopes. Individuals in this group were more likely to outgrow their allergy to cows' milk. The second phenotype is persistent allergy, and patients in this category have IgE directed against linear epitopes. Since baking does not destroy the linear epitopes, these patients did not tolerate baked milk products. Unlike individuals in the first phenotype group, patients in the persistent group were likely to experience CMA into adulthood.

While the results of this paper indicate that some CMA patients could consume a wider range of foods in their diets, these challenges should always take place under physician supervision. Of the 23 failed challenges to baked milk products, eight patients experienced anaphylactic reactions. In addition, the reported percentages of baked milk-tolerant patients may not be the same in the overall cows' milk allergic population as the researchers excluded subjects who had a recent history of a reaction to a baked-milk product.

In a similar study, researchers used the same food matrices and processing conditions but evaluated the effects on egg allergenicity (Lemon-Mulé *et al*., 2008). Of the 117 patients who were challenged, 87 tolerated the baked muffins and waffles containing egg (approximately 2.2 g of egg protein in each product). However, of the 62 baked egg-tolerant individuals challenged with scrambled eggs, only 23 were tolerant to these more mildly heated eggs.

For both milk and egg, the preceding studies seem to indicate that the degree of thermal processing is an important factor influencing the allergenicity of the resulting products. While relatively mild processing such as pasteurization of milk or cooking of scrambled egg does not seem to reduce allergenicity, more intense thermal processing

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can reduce or eliminate reactivity for some patients. For these two particular allergenic foods, the idea that the mild processes described do not largely change the allergenicity does not come as a surprise. Individuals, especially children, with cows' milk or egg allergy are relatively unlikely to consume raw milk or egg products due to the microbiological hazards associated with these products. The allergic reactions experienced by these individuals probably occurred after the ingestion of products that have undergone some kind of thermal processing. In addition to the degree of processing, the fact that the allergenic foods were baked in the context of a wheat-based food matrix could have an impact on allergenicity. These studies also reinforce the idea that the effects of allergenicity are patient specific, as some individuals reacted to the baked milk and egg products while others did not.

When SPTs are used to evaluate the effect of heating on allergenicity, the degree of processing, patient variability, and protein solubility can play important roles in the results. An oft-cited study examined the SPT reactivity of boiled milk in CMA patients (Norgaard *et al*., 1996). All the patients had positive SPTs to fresh milk and varying reactivity to individual milk proteins. Boiling the milk for 10 min (but not 2 min) eliminated positive SPTs in individuals sensitized to only BSA or BLG. However, subjects sensitized to whole casein had equal reactions to boiled and fresh milk. Based on the procedural information given for this study, it is difficult to know whether the negative SPTs with BSA and BLG were due to decreased allergenicity or decreased solubility of the allergens in the material used for the SPT.

6.7 OTHER MECHANISMS

In addition to immediate hypersensitivity, cows' milk has been implicated as a causative agent in several delayed hypersensitivity syndromes. These reactions are primarily cell-mediated and typically result in symptoms associated with one or more parts of the gastrointestinal tract. Two of the more important conditions are food protein-induced enterocolitis syndrome (FPIES) and eosinophilic gastrointestinal disorders (EGIDs).

FPIES primarily affects infants and symptoms include profuse vomiting and diarrhea 1–3 hours after ingestion of the offending food (Sampson *et al*., 2001; Sicherer, 2003; Sampson, 2004). In severe cases, the syndrome can include dehydration, lethargy, acidosis, and methemoglobinemia (Sicherer, 2003). The diagnostic criteria for FPIES include negative results for other conditions such as infections and IgE-mediated allergy, improvement following removal of the offending protein from the diet, positive oral challenge resulting in vomiting or diarrhea, markers of gastrointestinal inflammation including heme and eosinophils in the stool, and increased levels of peripheral polymorphonuclear leukocytes (>3500/L) (Sampson *et al*., 2001). Most patients outgrow FPIES by 2–3 years of age, but some maintain sensitivity into childhood (Sicherer, 2003).

Cows' milk protein is the most common trigger for FPIES, but approximately 50% of infants with the syndrome also react to soy proteins (Sicherer, 2003). In addition, some solid foods, such as oats, have been found to be responsible for the symptoms of FPIES in certain patients (Nowak-Wegrzyn *et al*., 2003). Treatment involves removal of offending foods from the diet.

Patients with FPIES typically do not have detectable levels of food-specific IgE (Sampson *et al*., 2001; Sicherer, 2003, 2005; Shek *et al*., 2005). Instead, this disorder appears to arise as the result of increased production of inflammatory cytokines by T cells in response to certain food proteins. Increased secretion of tumor necrosis factor (TNF)-α, interleukin (IL)-5, and IL-13 have been observed with cows' milk protein-specific T cells (Beyer *et al*., 2002; Sicherer, 2005). In addition these T cells produce almost no IL-10 and transforming growth factor (TGF)-β, anti-inflammatory cytokines associated with development of oral tolerance (Beyer *et al*., 2002).

Cows' milk is also often associated with EGIDs, including eosinophilic esophagitis and eosinophilic gastroenteritis (Sicherer, 2003; Sampson, 2004). EGIDs are generally characterized by inappropriate accumulation of eosinophils in one or more sections of the gastrointestinal tract without a known cause such as drug reactions or parasitic infections (Rothenberg, 2004). Symptoms observed with these syndromes include postprandial nausea, dysphagia, abdominal pain, vomiting, and diarrhea (Sicherer, 2003; Rothenberg, 2004; Sampson, 2004; Kapel *et al*., 2008). EGIDs, especially eosinophilic esophagitis, are more predominant in men than women, with male patients accounting for 70–75% of cases (Kapel *et al*., 2008; DeBrosse & Rothenberg, 2008).

While the precise pathogenesis of these disorders remains unclear, genetic and environmental factors have been identified, including a link to food allergy (Rothenberg, 2004). Cows' milk-specific IgE is commonly found in patients with cows' milk-induced eosinophilic gastroenteritis and SPT can be useful in identifying causative foods for EGIDs, but symptoms typical of immediate hypersensitivities (e.g., anaphylaxis) are not generally observed in these patients (Rothenberg, 2004; Shek *et al*., 2005; Spergel *et al*., 2002, 2007). Removal of the suspected offending foods frequently results in the resolution of symptoms, but steroids can also be incorporated into a treatment plan (Rothenberg, 2004; Spergel *et al*., 2002, 2007; DeBrosse & Rothenberg, 2008).

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7
Milk Carbohydrates and Oligosaccharides

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7.1 INTRODUCTION

Milk contains a plethora of components that encode functional health benefits to consumers far beyond that expected based on the nutritional content alone. Carbohydrates, and in particular the oligosaccharide, fractions are one of these components. Although the most dominant carbohydrate in mammalian milk is generally lactose, Gal(β1→4)Glc, which usually constitutes more than 80%, oligosaccharides are the third largest solid component within human milk following lactose and lipids, with concentrations up to 50 g/L or more in colostrum and an average of 10–15 g/L in mature milk (Kunz & Rudloff, 2002; Messer & Urashima, 2002). Compared with human milk, the concentration of oligosaccharides in the milk of the most relevant domestic animal is smaller by a factor of 10–100 (Boehm & Stahl, 2003) and proteins are the third component after lactose and fat (Gopal & Gill, 2000). The milk of monotremes and marsupials contains considerably more oligosaccharides than lactose. Among placentals (Eutheria), the milk of several species of the order Carnivora (Ursidae, Phocidae, Procyonidae, and Mustelidae) contains a relatively high ratio of milk oligosaccharides to lactose (Urashima *et al*., 2011a). Considering the three infraclasses of mammals (monotremes, marsupials and placentals), they have in common the fact that their young are suckled on milk produced from the mammary glands and, with few exceptions, the milk contains lactose in either free or bound form (Messer & Urashima, 2002).

7.2 LACTOSE AND MINOR SUGAR

Lactose is synthesized within lactating mammary gland by lactose synthase, a complex of a β4-galactosyltransferase I and α-lactalbumin, one of the whey proteins. Lactose is the principal energy source for the infant; when the young consume the mother's milk the lactose is split into galactose and glucose by intestinal lactase, which is located in the membrane of the microvilli of the brush border of the small intestine, and the monosaccharides are transported into the enterocyte by a specific mechanism. Glucose enters the circulation and is used as an energy source, while most of the galactose is converted to glucose in the liver to be used as an energy source as well (Messer & Urashima, 2002; Venema, 2012).

Lactose is the principal carbohydrate in most milk; its rate of synthesis in the epithelial cells of the mammary gland serves as a major factor influencing milk volume by maintaining its osmolarity and thus lactose is a very stable component of milk (Cant *et al*., 2002). Increase or decrease in lactose synthesis results in a change in milk volume but no change in protein or fat yield.

Many microorganisms thrive in milk by making use of the lactose as their main carbon source and fuel for growth. In yogurt and kefir (the most common products obtained from milk through fermentation), lactic acid bacteria convert lactose into lactic acid, which gives yogurt and kefir their characteristic acidic taste. On the other hand, the characteristic creamy consistency of fermented milk products is also due to the formation of lactic acid, since

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casein, the major protein constituent of milk, is insoluble at low pH (Adam *et al*., 2004).

Cheese whey is the aqueous fraction of milk that is generated in large quantities as a by-product in the process of cheesemaking. Every kilogram of cheese that is produced leaves behind nearly 10 L of the by-product. Therefore, huge amounts of whey and consequently lactose are available worldwide. Whey contains about 50% of the nutrients originally present in milk and whenever possible it is processed and used as a nutritional ingredient in animal feeds and food products (Adam *et al*., 2004). Lactose is the main solute of whey, present at a concentration of about 5%. Sometimes it is recovered by desiccation, but this procedure is expensive and often unprofitable, since it consumes high amounts of energy. Lactose from whey concentrates is used in bakery, dairy, and confectionery products, and also in infant formula. Lactases are often added to hydrolyze the disaccharide, which helps prevent problems caused by the frequent intolerance to the sugar. An alternative use of lactose is its conversion into biomass, ethanol, and other fermentative products (Adam *et al*., 2004). Some of the lactose derivatives that are manufactured industrially are widely used in food and in the pharmaceutical fields due to their special characteristics (Seki & Saito, 2012).

In humans, lactose intolerance caused by loss of lactase activity is a very common problem, with over 75% of adults worldwide developing lactose intolerance (Adam *et al*., 2004). As milk is a major component in the human diet, lactose intolerance limits the use of a valuable nutritional source for many people. Since milk is a source of the required calcium for maintaining bone health, lactose intolerance can also be associated with osteopenia in later life. The review of Brown-Esters *et al*. (2012) discusses dietary and biological factors that influence lactose digestion in humans and the work of Sabikhi (2004) illustrates methods of lactose reduction in bovine milk.

Carbohydrates other than lactose found in farm animals include oligosaccharides, glycopeptides, glycoproteins, and nucleotide sugars in small amounts. Nucleotide sugars in milk are of particular interest, since they are the glycosyl donors for glycosyltransferase in milk and mammary gland and are the precursors of glycoproteins, glycolipid, and oligosaccharides in the biosynthesis of milk. Nucleotides, nucleosides, and nucleobases occurring in the sub-milligram range per liter belong to the non-protein nitrogen (NPN) fraction of milk. The species-specific concentration pattern of milk constituents shows the species-specific physiological relevance of these minor compounds for the neonate (Schlimme *et al*., 2000). Nucleosides and nucleotides are used by the body as exogenous trophochemical sources and are not only active as metabolites but, furthermore, seem to be important in the regulation of body functions. A description of nucleotides as factors influencing the nutritional and health profile of milk and milk products can be found in Michaelidou (2008) and Gill *et al*. (2011).

7.2.1 Composition and concentration of carbohydrate in milk and dairy products of different species

All milks contain the same kind of constituents but in varying amounts. Within a given species, the quantity of milk produced and its chemical composition and physical properties are influenced by genetic (breed and genotype), physiological (age, lambing, body weight, number of lambs, stage and number of lactation), environmental, and management factors, and by method of milking (Bencini & Pulina, 1997; Antunac *et al*., 2001).

Different regions around the world have adapted the particular species common to their area for the purpose of producing milk: camel milk is produced in many countries in both Asia and Africa, horse milk is commonly consumed in Mongolia, and yak milk is used in Tibet. The latest nutritional discovery is donkey milk, which is exceptionally similar to human milk in terms of protein composition and is thought to be less prone to cause allergy. Currently, global milk production is dominated by five animal species: dairy cattle, buffalo, goats, sheep, and camels (Barłowska *et al*., 2011).

The concentration of lactose in milk from humans and different dairy species are summarized in Table 7.1. Following a meta-analysis of literature data, Barłowska *et al*. (2011) reported the average value of basic milk components for the five species of greatest importance in world milk production. In the paper from Mahmood and Usman (2010), the lactose content of buffalo milk was shown to be higher than that in cow and goat milk at a highly significant level ($P < 0.001$). When comparing the lactose content of buffalo and sheep milk, a moderately significant ($P < 0.01$) difference was obtained. There was a non-significant ($P > 0.05$) difference between the lactose content in cow, goat, and sheep milk. Park *et al*. (2007) reported data obtained in a previous work (and thus prior to the literature considered by Barłowska *et al*., 2011) but similar differences between the lactose content in cow, goat, sheep and human milk were noted; the lactose content of goat milk is less than that of cow and sheep milk.

Lactose in dairy cows is affected by mastitis. In an udder with a high somatic cell count, lactose concentration is lower and exhibits fluctuations, and hence measurements of lactose may be useful when evaluating disturbances in udder health (Forsbäck *et al*., 2010).

In the report from Bhosale *et al*. (2009) on goat milk, the highest concentration of lactose was observed in the first lactation (4.88 g/dL), with decreasing concentrations in

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Table 7.1. Lactose content in milk and colostrum of different mammalian species (mean value ± SD if present).

Species	Lactose (g/L)	Reference
Buffalo		
Milk	4.8 ± 0.1	Cataldi *et al*. (2003)
Milk	4.79 ± 0.068	Barłowska *et al*. (2011)
Milk	5.41 ± 0.54	Mahmood & Usmanet (2010)
Milk	4.9	Tamime & Robinson (2007)
Camel		
Colostrum	4.44	Zhang *et al*. (2005)
Colostrum	4.99	Fukuda *et al*. (2010)
Milk	3.63	Konuspayeva *et al*. (2010b)
Milk	4.24	Zhang *et al*. (2005)
Milk 3 weeks	5.74	Fukuda *et al*. (2010)
Milk 31 weeks	2.56	Konuspayeva *et al*. (2010a)
Milk	4.54	Konuspayeva *et al*. (2010a)
Milk	4.30 ± 0.078	Barłowska *et al*. (2011)
Milk	4.1	Tamime & Robinson (2007)
Cow		
Colostrum	1.4	Nakamura *et al*. (2003)
Colostrum	3.05	Fukuda *et al*. (2010)
Milk	4.2	Nakamura *et al*. (2003)
Milk	5.17	Fukuda *et al*. (2010)
Milk	4.1 ± 0.1	Cataldi *et al*. (2003)
Milk	4.82 ± 0.021	Barłowska *et al*. (2011)
Milk	4.8	Fox & McSweeney (1998)
Milk	4.7	Park *et al*. (2007)
Milk	4.51 ± 0.38	Mahmood & Usmanet (2010)
Milk	4.6	Martinez-Ferez *et al*. (2006)
Donkey		
Milk	6.88/6.73/5.87*	Barłowska *et al*. (2011)*
Milk	7.4	Fox and McSweeney (1998)
Milk	6.33	Guo *et al*. (2007)
Goat		
Milk	5.2 ± 0.1	Cataldi *et al*. (2003)
Milk	4.51 ± 0.026	Barłowska *et al*. (2011)
Milk	4.1	Park *et al*. (2007)
Milk	4.39 ± 0.34	Mahmood & Usmanet (2010)
Milk	4.6	Tamime & Robinson (2007)
Milk	4.5	Martinez-Ferez *et al*. (2006)
Human		
Colostrum	5.60	Coppa *et al*. (1993)
Colostrum	5.5	Kunz *et al*. (1999a)
Colostrum	5.03	Thurl *et al*. (2010)
Milk	6.25–6.89	Coppa *et al*. (1993)
Milk	6.8	Kunz *et al*. (1999a)
Milk	5.7–6.0	Thurl *et al*. (2010)
Milk	7.0	Fox & McSweeney (1998)
Milk	6.9	Park *et al*. (2007)
Milk	6.8	Martinez-Ferez *et al*. (2006)
Mare		
Milk	6.90/6.91/6.74*	Barłowska *et al*. (2011)*
Milk	6.2	Fox & McSweeney (1998)
Sheep		
Milk	4.1 ± 0.1	Cataldi *et al*. (2003)
Milk	4.75 ± 0.035	Barłowska *et al*. (2011)
Milk	4.8	Fox & McSweeney (1998)
Milk	4.9	Park *et al*. (2007)
Milk	4.77 ± 0.31	Mahmood & Usmanet (2010)
Milk	4.8	Martinez-Ferez *et al*. (2006)

*For donkey, mare and yak, non-averaged data from the different cited papers are reported.

the second (4.72 g/dL), third (4.50 g/dL), and fourth (4.19 g/dL) lactations; higher concentrations of lactose were found at the beginning of each lactation compared with the middle of lactation. The same trend was observed in Travnik sheep milk, where lactose content was highest at the beginning of lactation (4.97%) and lowest at the end of lactation (4.09%) (Pavic *et al*., 2002). Dario *et al*. (1996) also reported a higher lactose content at the beginning (5.32%) compared with the end (4.93%) of lactation for milk taken from Leccese sheep.

In camels, Fukuda *et al*. (2010) and Konuspayeva *et al*. (2010a) reported an increase in milk lactose content during lactation, whereas Zhang *et al*. (2005) have shown a decreasing lactose content (Table 7.1). Coppa *et al*. (1993) studied the change in carbohydrate composition of human milk during 4 months of lactation, and showed that the mean lactose concentration increased from 5.6% in colostrum to 6.89% on day 120. The same increase in lactose content during the first 3 weeks of human lactation has been reported by Thurl *et al*. (2010). Table 7.2 shows the content of total sugar or lactose in some dairy products from different species.

The concentration of minor sugars in milk of dairy species is shown in Table 7.3. Interestingly, milk from bovine and buffalo contained significantly more *N*-acetylhexosamines than other mammals. With regard to

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Table 7.2. Approximate composition of sugar (%) in some dairy products.

Product	Sugar*	Lactose	Reference
Light whipping cream	3.0		Fox & McSweeney (1998)
Butter	0.06		Fox & McSweeney (1998)
Anhydrous oil	0.0		Fox & McSweeney (1998)
Ice-cream†	23.8		Fox & McSweeney (1998)
Evaporated whole milk	10.0		Fox & McSweeney (1998)
Sweetened condensed milk	54.4		Fox & McSweeney (1998)
Whole milk powder	38.4		Fox & McSweeney (1998)
Skim milk powder	52.0		Fox & McSweeney (1998)
Whey powder‡	74.5		Fox & McSweeney (1998)
Casein powder	0.0		Fox & McSweeney (1998)
Cottage cheese, creamed	2.7		Fox & McSweeney (1998)
Quarg	3.0		Fox & McSweeney (1998)
Camembert cheese	0.5		Fox & McSweeney (1998)
Blue cheese	2.3		Fox & McSweeney (1998)
Cheddar cheese	1.3		Fox & McSweeney (1998)
Parmesan cheese	3.2		Fox & McSweeney (1998)
Mozzarella cheese	2.2		Fox & McSweeney (1998)
Processed cheese§	1.6		Fox & McSweeney (1998)
Acid whey	4.2		Fox & McSweeney (1998)
Koumisse¶		5.5	Uniacke-Lowe *et al*. (2010)
Kurut‖		2.34	Zhang *et al*. (2008)
Apulian cacioricotta cheese**		2.3	Pasqualone *et al*. (2003)
Ricotta cheese**		2.9	Pizzillo *et al*. (2005)
Pressed cheese**		0.17	Trujillo *et al*. (1999)
Valencay cheese**		1.8	Hosono & Sawada (1995)
Crottin de Chavignol cheese**		0.7	Hosono & Shirota (1994)

*Total carbohydrate.
†Hardened vanilla, 19% fat.
‡Cheddar (sweet) whey.
§American pasteurized processed cheese.
¶A product from equine milk.
‖A product from yak milk.
**Some goat milk cheeses.

milk derivatives, the raw materials used in processed cheese greatly affect the course of the cooking process and the main features of the final product, especially its appearance, flavor, texture and keeping quality. Browning in mozzarella surrogate is likely to be due to galactose undergoing Maillard reactions (Van Boekel, 1998). In an analysis of mozzarella cheese manufactured in Italy (known as "pasta filata") from bovine and buffalo milk, Cataldi *et al*. (2003) found galactose concentrations of 5–7 mg/100 mL of aqueous extract; the concentration of sugars in bovine milk whey permeate found by the same authors is reported in Table 7.3. This minor sugar profile is similar to that obtained for milk samples, suggesting the relatively low impact of the cheesemaking processes, at least when the soluble fraction of milk is considered.

Cow, goat and sheep colostrum contain significant amounts of nucleotides, which increase from the moment of parturition to reach a maximum 24–48 hours later. The nucleotide concentration decreased thereafter with advancing lactation (Gil & Sanchez-Medina, 1981). The same trend was shown by Gill *et al*. (2011) for bovine milk. Cows' milk contains substantial amounts of orotic acid that increase during lactation, whereas there is no such increase in goat and sheep milk (Gil & Sanchez-Medina, 1981). Orotic acid is not present in human milk, and ruminant milk principally contains adenosine monophosphate,

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Table 7.3. Minor sugars and nucleotide content in milks and/or derivatives of different mammalian species.

	Milk									Milk derivatives (pasta filata)	Whey permeate	
Species	**Galactose (mg/dL)**	**Glucose (mg/dL)**	**GalNAc (mg/dL)**	**Monosaccharides (g/L)**	**5′-AMP***	**5′-CMP***	**5′-GMP***	**5′-UMP***	**Orotic acid**	**Galactose (mg/dL)**	**Galactose (mg/dL)**	**Glucose (mg/dL)**
Buffalo	3.32 ± 0.05[†]	2.72 ± 0.07[†]	5.09 ± 0.05[†]							5		
Cow	4.04 ± 0.09[†]	2.52 ± 0.07[†]	6.57 ± 0.07[†]		2.03[‡]	1.9[‡]	n.d.[‡]	n.d.[‡]	26.8[‡]	7	1.05	0.58
					n.d.[§]	3.3 ± 0.1[§]	n.d.[§]	0.5 ± 0.2[§]				
Goat	0.56 ± 0.05[†]	1.55 ± 0.07[†]	<0.5[†]		6.73[‡]	5.4[‡]	n.d.[‡]	14.5[‡]	10.2[‡]			
					2.4 ± 0.2[§]	3.6 ± 0.2[§]	9.4 ± 0.5[§]	37.2 ± 0.8[§]				
Sheep	0.31 ± 0.02[†]	0.32 ± 0.05[†]	<0.5[†]		7.5[‡]	8.7[‡]	n.d.[‡]	19.2[‡]	3.3[‡]			
					12.1 ± 0.0[§]	5.7 ± 0.3[§]	6.3 ± 0.0[§]	187.4 ± 4.4[§]				
Human					2.0[¶]	1.9[¶]	0.3[¶]	1.3[¶]				
4 days				0.94[‖]								
30 days				0.77[‖]								
120 days				0.47[‖]								

*AMP, adenosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; GMP, guanosine 5′-monophosphate; UMP, uridine 5′-monophosphate (reported as μmol/dL).
[†]Value taken from Cataldi *et al*. (2003). Mean value ± SD, $N = 3$.
[‡]Value taken from Gil & Sanchez-Medina (1981).
[§]Value taken from Gill *et al*. (2012).
[¶]Value taken from Gil & Sanchez-Medina (1982).
[‖]Value taken from Coppa *et al*. (1993).
n.d., not detectable.

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cytidine monophosphate, and uridine monophosphate (Table 7.3); detailed nucleotide concentrations during lactation of humans and dairy species have been reported by Michaelidou (2008). In sheep and goats, Plakantara *et al.* (2010) showed that the total nucleotide content was 294–441 μmol/L in ovine milk and 166–366 μmol/L in caprine milk. The nucleotide content in colostrum (1–3 days) of both species was higher than in mature milk (15 days), and uridine monophosphate was the dominant nucleotide in all samples. Gill *et al.* (2012) reported the concentration of the total potentially available nucleosides in bovine, caprine and ovine milk and showed that ovine milk contained the highest concentration of each of the different nucleotides while bovine milk contained the lowest (Table 7.3). Since the nucleotide concentrations in caprine milk have been favorably compared with those in human milk (Prosser *et al.*, 2008), supplementation of caprine milk-based infant formula with nucleotides is not necessary as such products provide similar quantities of free nucleotides to those in nucleotide-supplemented bovine milk-based infant formula (Gill *et al.*, 2012).

7.3 OLIGOSACCHARIDES

Even though the biomolecular and biomedical study of glycobiology is undergoing a revolution (Finkelstein, 2007), there is limited knowledge on the metabolism of oligosaccharides in mammalian milk. Oligosaccharides are defined as carbohydrates that are synthesized in the mammary gland and contain between 3 and 10 monosaccharides covalently linked through glycosidic bonds and can be divided into neutral and acidic fractions. The building blocks of milk oligosaccharides are the five monosaccharides D-glucose (Glc), D-galactose (Gal), *N*-acetylglucosamine (GlcNAc), L-fucose (Fuc), and sialic acid [Sia; *N*-acetylneuraminic acid (Neu5Ac) in humans and both Neu5Ac and *N*-glycolylneuraminic acid (Neu5Gc) in most other species]. Lactose, Gal(β1→4)Glc, forms the reducing end of milk oligosaccharides. Gal in lactose can be sialylated in α2→3 and/or α2→6 linkages to form 3′-sialyllactose (3′-SL) and 6′-sialyllactose (6′-SL), respectively. Lactose can also be fucosylated in α1→2 and α1→3 linkages to form 2′-fucosyllactose (2′-FL) and 3′-fucosyllactose (3′-FL), respectively. These trisaccharides are called the short-chain milk oligosaccharides. To form more complex milk oligosaccharides, lactose is elongated with up to 15 *N*-acetyllactosamine repeat units, i.e., Gal(β1→3/4)GlcNAc. Lactose or the polylactosamine backbone can be sialylated in α2→3 and/or α2→6 linkages and/or fucosylated in α1→2, α1→3, and/or α1→4 linkages (Bode, 2006, 2009). In human milk the linkage of fucose is genetically connected to the secretor/Lewis blood group status of the individual mother (Kunz *et al.*, 2000; Boehm & Stahl, 2007).

Most human milk oligosaccharides (HMOs) are resistant to digestion and absorption within the small intestine (Engfer *et al.*, 2000; Gnoth *et al.*, 2000) and reach the infant colon where they can act as prebiotics that stimulate the growth of beneficial microorganisms such as various species of *Bifidobacterium*. They can also act as receptor analogs that inhibit the attachment of harmful microorganisms to the infant's colonic mucosa. A small part of the milk oligosaccharides are absorbed intact into the circulation (Gnoth *et al.*, 2001) and through selectin binding they behave as anti-inflammatory mediators by reducing the binding of platelets to neutrophils. The sialic acid of sialylated milk oligosaccharides can be absorbed and utilized as a precursor for the biosynthesis of brain gangliosides and sialoglycoprotein (Sakai *et al.*, 2006). Many of the physiological effects observed for the bioactive components of milk have only been proven *in vitro* or in animal models.

The colostrum of cows and other domestic farm animals is a potential source of free oligosaccharides that, if isolated, can be utilized as functional foods or animal feedstuffs on an industrial scale. Several reports have suggested that bovine milk oligosaccharides can be used as anti-infection resources (Angeloni *et al.*, 2005) and that it may be feasible to use sialyllactose, separated from cheese whey or bovine colostrum, as a functional food for brain activation (Sakai *et al.*, 2006).

The variation between bovine milk oligosaccharides (BMOs) and HMOs is extensive and studies indicate that the diversity and vast number of HMOs are essential to the overall health of the infant (Mills *et al.*, 2011). Strategies to emulate the oligosaccharide content of human milk are ongoing (Espinosa *et al.*, 2007); currently, infant formula enriched with fructooligosaccharides and glucooligosaccharides have proven successful as prebiotic ingredients in stimulating bifidobacteria and lactobacilli in the gut (Moro *et al.*, 2002) but lack the other important functions described for oligosaccharides.The highest priority for infant formula is the search for the structural elements of HMO that are considered crucial to their biological effect and would serve as a scientific basis for the selection of oligosaccharides from sources other than human. Because synthetic oligosaccharides are rare and expensive, and human milk is not available for large-scale oligosaccharide purification, we need to find alternative sources from which to obtain sufficient amounts.

BMOs are particularly attractive candidates because the large size of the existing bovine dairy industry positions them as a readily available source for significant amounts of oligosaccharides and because they have biological functions close to HMO. Most of these oligosaccharides are lost in the whey during cheesemaking and exploitation

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of this potential source may add value and encourage a decrease in environmental pollution. Barile *et al.* (2010) showed that whey permeate, a by-product obtained when cheese whey is passed through an ultrafiltration membrane, could be a source of oligosaccharides with compositions similar to those present in human milk.

Interestingly, goat milk contains a significant concentration and variety of oligosaccharides, similar to those in human milk, and may also hold potential as a natural source of lactose-derived oligosaccharides as a supplement for infant formula and for the development of functional foods, having been successfully isolated by membrane technology (Martinez-Ferez *et al*., 2006, 2008). In a preliminary work on goat whey, Oliveira *et al.* (2010) demonstrated the presence of an oligosaccharide mixture, partly in the permeate and in the final retentate, after processing with a tighter ultrafiltration membrane.

7.3.1 Purification and characterization of oligosaccharides from milk

Studies on human and bovine milk oligosaccharides have been performed for over 50 years, especially in the field of structural analysis. To date the structures of 115 HMOs have been characterized (Urashima *et al*., 2011b); 21 further oligosaccharides with a decaose core have been characterized using matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOFMS) in negative ion mode (Amano *et al*., 2009); and around 200 oligosaccharides have been detected using microfluidic high-performance liquid chromatography (HPLC) chip mass spectrometry (Niñonuevo *et al*., 2006). Oligosaccharides are classified into 13 core groups. The main structural features of HMOs are the presence of type I oligosaccharides containing Gal(β1→3) GlnNAc (lacto-Nbiose I) and type II oligosaccharides that contain Gal(β1→4)GlcNAc (N-acetyllactosamine); type I oligosaccharides predominate over type II. The milk oligosaccharides of other species mostly have the type II but not the type I unit (Urashima *et al*., 2001), exceptions being chimpanzee, bonobo, and orang-utan, whose milk or colostrum contains both types I and II with type II predominant over type I (Urashima *et al*., 2009). Domestic animal milk, a widely available resource, can offer oligosaccharides with structures or functions similar to those of human milk.

The structures of 25 varieties of BMO have been characterized (Urashima *et al*., 2011b), while 39 oligosaccharides have been detected in bovine colostrum by microchip liquid chromatography separation and high-performance mass spectroscopy including Fourier transform ion cyclotron resonance (FTICR) and time-of-flight analysis (Tao *et al*., 2008). Using the latter methodology, recently Tao *et al*. (2010) identified 29 distinct porcine milk oligosaccharides (PMO).

Milk or colostrum oligosaccharides of the following species have been studied and characterized: platypus (monotreme), echidna (monotreme), tammar wallaby (marsupial), cow, buffalo, horse, goat, sheep, dog, pig, Ezo brown bear, Japanese black bear, polar bear, giant panda, white-nosed coati, mink, crabeater seal, hooded seal, harbor seal, bearded seal, minke whale, beluga, bottlenose dolphin, Bryde's whale, sei whale, Asian elephant, African elephant, rat, brown capuchin, giant anteater, hyena, chimpanzee, bonobo, gorilla, orang-utan, siamang, and camel (Fukuda *et al*., 2010; Tao *et al*., 2010; Urashima *et al*., 2011a).

7.3.2 Methods for structural analysis

Carbohydrates are water-soluble compunds, and generally they lack groups necessary for ultraviolet and fluorescence detection (Cataldi *et al*., 2003). High-pH anion-exchange chromatography (HPAEC) allows high resolution of closely related carbohydrates, and in conjunction with pulsed amperometric detection is one of the most suitable methods for carbohydrate analysis because it is quantitative and does not need pre- or post-column derivatization (Thurl *et al*., 1996). These analytical techniques have proven to be efficient and simple; however, derivatization prior to separation usually results in higher sensitivity. Analysis of derivatized milk glycans by normal-phase HPLC (Charlwood *et al*., 1999), reversed-phase HPLC (Charlwood *et al*., 1999; Warren *et al*., 2001; Asakuma *et al*., 2007) and also by capillary electrophoresis (Nakajima *et al*., 2006; Bao & Newburg, 2008) has been efficient.

Nuclear magnetic resonance (NMR) spectroscopy is the most important technique that provides sequence information, including linkage and α/β-anomeric configurations. Urashima and coworkers isolated various oligosaccharides from many mammalian species' milk/colostrum and characterized their structural features (Urashima *et al*., 1991; Kogelberg *et al*., 2004). However, it is often difficult to assign the branching pattern using only the NMR technique. In recent years mass spectrometry has been an indispensable technique for structural analysis of oligosaccharides and useful for the analysis of higher oligosaccharides with high sensitivity. The combination of liquid chromatography and time-of-flight mass spectrometry using the HPLC chip technology has been extensively and successfully used for oligosaccharide profiling in human milk (Niñonuevo *et al*., 2006), bovine milk (Tao *et al*., 2008; Barile *et al*., 2010), and porcine milk (Tao *et al*., 2010).

Even mass spectrometry methods often cannot differentiate isomeric branched or linear oligosaccharides such

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as lacto-*N*-neohexaose and *para*-lacto-*N*-hexaose (Galβ1→4GlcNAcβ1→3Galβ1→4Glc-NAcβ1→3Galβ1→4Glc). Thus it is still difficult to characterize anomeric configurations, branching configurations, and epimeric forms and this information is often obtained by analysis of the digestion products with specific exoglycosidases (Kinoshita *et al*., 2009). Mariño *et al*. (2011) have developed an analytical scheme for milk oligosaccharide profiling based on fluorescent labeling with 2-aminobenzamide, pre-fractionation by weak anionic exchange chromatography, and separation by hydrophilic interaction liquid chromatography (HILIC)–HPLC. The robustness of the methodology was demonstrated using bovine colostrum oligosaccharides as a case study.

7.3.3 Composition and concentration of oligosaccharides in milk of different species

The ratio of milk oligosaccharides to free lactose in milk/colostrum varies depending on the mammalian species. For example, in mature human milk, lactose and oligosaccharides constitute 80% and 20%, respectively, of the carbohydrate fraction, whereas mature bovine milk contains only trace amounts of oligosaccharides with a smaller number of structures (Urashima *et al*., 2001; Martinez-Ferez *et al*., 2006). Human and elephant milk contain the greatest concentrations of oligosaccharides with the greatest structural complexity (Kunz *et al*.,1999a). The milk of a few species such as monotremes (platypus and echidna) and marsupials (e.g., kangaroo, wallaby, possum, koala, wombat) and a few species of Carnivora (eutherians) such as the Canoidea (other than dog) contains greater amounts of oligosaccharides than of lactose (Urashima *et al*., 2001, 2011a).

Mature human milk and colostrum contain 12–13 g/L and 22–24 g/L of oligosaccharides, respectively (Kunz *et al*., 1999b; Boehm & Stahl, 2003) and the concentration of neutral oligosaccharides is greater than that of acidic oligosaccharides; the neutral fraction contains many fucosyloligosaccharides including significant amounts of 2′-FL, lacto-*N*-fucopentaose I, lacto-*N*-difucohexaose I, and lacto-*N*-tetraose (Thurl *et al*., 1996; Coppa *et al*., 1999; Kunz *et al*., 2000; Chaturvedi *et al*., 2001; Asakuma *et al*., 2008). The principal acidic oligosaccharides during days 1–3 of lactation are disialyllacto-*N*-tetraose, 6′-SL, 3′-SL and sialyllacto-*N*-tetraose c. 6′-SL predominates over 3′-SL in human/milk colostrum (Kunz *et al*., 2000; Martín-Sosa *et al*., 2003; Asakuma *et al*., 2007; Bao *et al*., 2007; Thurl *et al*., 2010). Bao *et al*. (2007) and Thurl *et al*. (2010) both demonstrated an increase in 6′-SL in the first 3 weeks of lactation. The variation in oligosaccharide concentration between studies may be due to differences between the quantification methods used, and can depend on the donor's ethnicity and on lactation stage at which the milk samples were obtained (Urashima *et al*., 2011a). Nevertheless, a significant decrease in the acidic sugar fraction as a whole and of most individual oligosaccharides is generally found during the first few months of lactation. Coppa *et al*. (1993) studied the change in carbohydrate composition in human milk during 4 months of lactation and showed that the oligosaccharide level decreased from 20.9 to 12.9 g/L (Table 7.4); monosaccharides (glucose and fucose) represented only 1.2% of total carbohydrate and decreased from 0.094% to 0.047%.

In contrast to human, bovine milk contains only trace amounts of milk oligosaccharides, but colostrum contains over 1 g/L of milk oligosaccharides with sialyl oligosaccharides constituting 70% of the total, of which those containing *N*-glycolylneuraminic acid (Neu5Gc) comprising less than 5%. Most of the acidic oligosaccharide fraction of bovine colostrum consists of 3′-SL, 6′-SL, 6′-sialyllactosamine (SLN) and disialyllactose (DSL), with 3′-SL constituting 70% of the total (Tao *et al*., 2008) (Table 7.4).

Gopal and Gill (2000) summarized the data available on the variety of oligosaccharides found in bovine colostrum. Martin *et al*. (2001) investigated the distribution of sialoglycoconjugates (expressed as glycoconjugate-bound sialic acid, oligosaccharides comprised) from four stages of cow lactation and found the highest values in the colostrum, these decreasing in transitional and mature milks and increasing again in late-lactation milk. Martín-Sosa *et al*. (2003) identified five sialyl oligosaccharides in bovine milk, of which 6′-SLN and 3′-SL were the most abundant and decreased during the course of lactation. Nakamura *et al*. (2003) monitored three sialyl oligosaccharides in bovine colostrum over a 7-day period and showed that the level of measured oligosaccharides was maximal immediately after parturition, rapidly decreasing by 48 hours postpartum (Table 7.4).

McJarrow and van Amelsfort-Schoonbeek (2004) measured the concentrations of 6′-SL, 3′-SL, 6′-SLN and DSL in the colostrum of individual Jersey and Friesian cows at first milking, individual Jersey and Friesian cows over the first five milkings, and also in colostrum pooled from Friesian or Jersey herds over the first 2 days after parturition. Their results show that at first milking the 6′-SL concentration was higher in Friesian colostrum ($P < 0.001$) while DSL ($P < 0.088$) and 3′-SL ($P = 0.107$) levels were higher in Jersey colostrum. The concentration of all measured sialyl oligosaccharides was highest in colostrum from the first milking after parturition, dropping rapidly in successive milkings (Table 7.4). The pooled colostrum samples of Friesian milk showed a higher proportion of 6′-SLN.

Barile *et al*. (2010) studied the oligosaccharide composition of bovine milk during the first 3 days of lactation and

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Table 7.4. Oligosaccharide concentration in colostrum and milk of different species.

Organism	Oligosaccharides (g/L)	3′-SL (mg/L)	6′-SL (mg/L)	6′-SLN (mg/L)	DSL (mg/L)	Reference
Human						
Colostrum	22–24					Kunz *et al.* (1999a); Boehm & Stahl (2003)
Colostrum		350	1310			Thurl *et al.* (2010)
Colostrums 1 day		362 ± 103	342 ± 120			Asakuma *et al.* (2007)
Colostrums 3 days		258 ± 80	396 ± 86			Asakuma *et al.* (2007)
Colostrum 4 days	20.9					Coppa *et al.* (1993)
Colostrum 4 days		82 ± 29	276 ± 99			Bao *et al.* (2007)
Mature milk 30 days	15.5					Coppa *et al.* (1993)
Mature milk 120 days	12.9					Coppa *et al.* (1993)
Mature milk	12–13	100–300	300–500			Kunz *et al.* (1999a); Boehm & Stahl (2003)
Mature milk		63 ± 14	306 ± 157			Bao *et al.* (2007)
Mature milk		240	490			Thurl *et al.* (2010)
Mature milk	5–8					Martinez-Ferez *et al.* (2006)
Mature milk	5–10					Kunz & Rudloff (2002)
Cow						
Colostrum		150	30	70	30	Parkkinen & Finne (1987)
Colostrum		354	147	210	135	Martín-Sosa *et al.* (2003)
Colostrum		261–867	92–243	97–239	166–283	McJarrow & van Amelsfort-Schoonbeek (2004)
Colostrum		853 ± 0.26	141 ± 0.06	117 ± 0.04	ND	Nakamura *et al.* (2003)
Colostrum (second milking)		1245 ± 82	85 ± 6	119 ± 7	126 ± 8	Fong *et al.* (2011)
Colostrum (fourth milking)		739 ± 53	73 ± 2	117 ± 10	80 ± 7	Fong *et al.* (2011)
Mature milk		94–119	67–88	145–176	41–77	Martín-Sosa *et al.* (2003)
Mature milk		35–50	14–25	9–12	2–7	McJarrow & van Amelsfort-Schoonbeek (2004)
Mature milk 7 days		30	25	12	ND	Nakamura *et al.* (2003)
Mature milk	0.03–0.06					Martinez-Ferez *et al.* (2006)
Skim milk		51 ± 4	6.3 ± 0.4	0.13 ± 0.02	1.5 ± 0.1	Fong *et al.* (2011)
Homogenized milk		48 ± 4	9.6 ± 0.8	0.1 ± 0.03	3.1 ± 0.2	Fong *et al.* (2011)
Unpasteurized milk		47 ± 4	3.6 ± 0.3	<LOD	0.54 ± 0.01	Fong *et al.* (2011)
Elephant						
Milk	21					Kunz *et al.* (1999b)
Goat						
Milk	0.25–0.30	30–50	50–70	1–5		Martinez-Ferez *et al.* (2006)
Milk	2.49					Viverge *et al.* (1997)
Sheep						
Milk	0.02–0.04					Martinez-Ferez *et al.* (2006)

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suggested that the variation in terms of oligosaccharide species and relative abundances between cows probably depended on unique animal genetic variability. They reported that in 5.8% of the samples sialyllactosamine was the second most abundant oligosaccharide following 3′-SL. Recently, Fong *et al*. (2011) quantified the different oligosaccharides in bovine milk, bovine colostrum, and infant formula and compared their data with that previously reported in the literature. Table 7.4 reports the concentrations of the acid oligosaccharide fraction in the milks of dairying species and, for comparison, in the human.

Among other dairy species, oligosaccharides are present in goat milk at higher levels than in bovine milk and contain fucosylated oligosaccharides (Nakamura & Urashima, 2004). The oligosaccharide content of goat milk is 0.25–0.39 g/L, which is higher than that of bovine (0.03–0.06 g/L) or ovine (0.02–0.04 g/L) milk and the variety of oligosaccharides is greater than that in bovine or ovine milk, as shown by the profiles from HPEAC analysis (Martinez-Ferez *et al*., 2006; Mehra & Kelly, 2006). Goat milk has a profile similar to human milk, with the presence of trace amounts of oligosaccharides derived from lacto-*N*-hexaose and also oligosaccharides containing *N*-acetylglucosamine.

In camels, the oligosaccharide concentration is higher in colostrum than in mature milk; the camel resembles the cow, with 3′-SL being the predominant acidic oligosaccharide, although different oligosaccharides have been found in camel and cow and when compared with the human (Fukuda *et al*., 2010).

Pig milk oligosaccharides resemble BMO in being predominantly sialylated but, unlike BMO, 6-fucose-containing PMO were detected, suggesting a closer relationship between PMO and HMO than between BMO and HMO (Tao *et al*., 2010).

7.4 CARBOHYDRATES AS PREBIOTICS IN THE GASTROINTESTINAL TRACT

Over the past 30 years it has become apparent that the colon is one of the most active organs metabolically of the human body, harboring an extremely complex microbial ecosystem that does not only act as a barrier against infection but also plays an active role in salvaging energy from non-digestible food ingredients that human enzymes cannot affect. Probiotics and prebiotics have been used in the past for manipulation of the microbiota. In 1995 Gibson and Robertfroid introduced the concept of prebiotics and Robertfroid *et al*. (2010) published reviews of research. Many attempts have been made to redefine the "prebiotic" concept and the current accepted definition remains closer to the 1995 concept: a dietary prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota thus conferring benefit(s) on host health (Gibson *et al*., 2010). Non-digestibility of prebiotic ingredients has been excluded from later definitions. However, to elicit an effect on the target site, the prebiotic must be either non-digestible or partially digestible to reach targets that are lower in the gastrointestinal tract. Currently, only three dietary ingredients have achieved prebiotic status in the European Union and fulfil all the above-presented criteria: fructooligosaccharides (and inulin), galactooligosaccharides, and lactulose (Kolida & Gibson, 2011). At the beginning of the twentieth century it was believed that the high acidity of the feces of breast-fed infants was due to the action of lactobacilli present in the colonic microflora; it was suggested that these lactobacilli digest milk carbohydrates, producing large amounts of lactic and acetic acids; the resulting acidity would inhibit the growth of harmful bacteria within the infant colon. It was found that the growth-stimulating factor of *Lactobacillus bifidus* occurs in human milk and was termed "bifidus factor" (Schönfeld, 1929). Afterwards Richard Kuhn collaborated with Paul György in order to clarify the structures of the actual components of the bifidus factor in human milk and found that they consist of a variety of oligosaccharides (György *et al*., 1954; reviewed in Urashima *et al*., 2011b).

Analysis of the fecal microbial communities indicated that host diet had a major influence on bacterial diversity (Ley *et al*., 2008). In breast-fed infants the intestinal microbiota is represented mainly by bifidobacteria that can metabolize the neutral oligosaccharide fraction of human milk, whereas in most formula-fed infants similar amounts of *Bacteroides* and bifidobacteria (~40%) were found. The minor components of the fecal samples from breast-fed infants were mainly lactobacilli and streptococci; samples from formula-fed infants often contained staphylococci, *Escherichia coli*, and clostridia (Harmsen *et al*., 2000). Several investigators have speculated that the difference in intestinal flora between breast-fed and formula-fed infants contributes to the functional benefits that breastfeeding has over formula feeding, i.e., inhibition of pathogen colonization (Knol *et al*., 2005), induction of oral tolerance to dietary allergens (Hanson & Telemo, 1997), and modulation of the systemic immunological and inflammatory responses.

In lactose-intolerant subjects, undigested lactose consumed as a component in milk and other dairy products arrives in the large intestine without being absorbed in the small intestine. This undigested lactose potentially serves as a prebiotic for stimulating bacterial growth (such as bifidobacteria and lactobacilli) that in turn generates non-digestible oligosacchardes, which are known to enhance calcium absorption in animals and humans (see Kwak *et al*., 2012 for a review on lactose influence on calcium absorption).

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Most of the HMOs are resistant to hydrolysis by intestinal lactase (β-galactosidase) or other brush border enzymes and there is evidence that the majority survive passage through the small intestine (Gnoth *et al*., 2001) and enter the colon where they are subject to fermentation by colonic bacteria (Brand-Miller *et al*., 1998; Newburg, 2000). To understand which oligosaccharides are utilized and by which metabolic pathway they are digested by bifidobacteria, two series of reports have been published based on different approaches: one is to determine the enzyme for the metabolism of HMOs by bifidobacteria, and the other is to investigate the consumption of each component of HMO during *in vitro* fermentation by bifidobacteria (summarized in Urashima *et al*., 2011a). Ward *et al*. (2006) showed that the strain *Bifidobacterium infantis* can grow on purified HMOs as sole carbon source and, in a follow-up study, *Bifidobacterium longum* biovar *infantis* was shown to achieve the highest cell density, suggesting that HMOs may even selectively promote the growth of certain bifidobacteria strains (Ward *et al*., 2007). LoCascio *et al*. (2007) demonstrated that the infant gut isolate *B. longum* biovar *infantis* ATCC 15697 possessed fucosidase and sialidase activities not present in other tested strains and preferentially consumed small-mass oligosaccharides, representing 64% of the total HMOs available. The *B. longum* subsp. *infantis* ATCC 15697 genome was fully sequenced to permit investigation into the molecular mechanism underlying this phenotype and a novel 43-kb genomic cluster dedicated to the uptake and processing of HMOs was identified (Sela *et al*., 2008). LoCascio *et al*. (2010) performed comparative genomic hybridization analyses to associate genotypic biomarkers among 15 *B. longum* strains exhibiting various HMO utilization phenotypes and host associations; the results showed that these strains could be differentiated into at least two distinct subspecies: *B. longum* subsp. *infantis*, specialized to utilize milk carbon, and *B. longum* subsp. *longum*, specialized for plant-derived carbon metabolism.

7.5 OTHER OLIGOSACCHARIDE FUNCTIONS

The biological functions of milk oligosaccharides have recently been an active field of research. There is considerable evidence that virulent enteric bacteria and viruses initiate infection by binding to particular sugar chains of glycolipids and glycoproteins on the surface of cells of the mucous epithelium of the digestive and respiratory tracts (Sharon, 1996). Because of their structural mimicry of the sugar chains of glycoproteins on the mucous membrane, Newburg (1997) suggested that addition of several oligosaccharides or glycoconjugates to milk formula would protect infants against infectious disease. Several *in vitro* studies based on cultured differentiated epithelial cells showed that HMOs can protect breast-fed infants against infections by blocking the adhesion of pathogens (Bode, 2006). Morrow *et al*. (2005) suggested that HMOs containing Fuc(α1→2)Gal inhibit the attachment of the toxic protein STa, produced by pathogenic *E. coli*, to colonic epithelial cells. Ruiz-Palacios *et al*. (2003) found that the α1→2-linked fucosylated glyconjugates expressed in human milk inhibit binding of *Campylobacter* to intestinal cells. Other research has reported the anti-pathogenic effects of different HMOs against various bacteria and viruses (Le Pendu, 2004; Perret *et al*., 2005; Coppa *et al*., 2006). It has been reported that the HMO fraction reduces HIV-1 gp120 binding to dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) in human dendritic cells by more than 60% (Hong *et al*., 2009). Thorven *et al*. (2005) showed that the human secretor *FUT2* gene, which codes for an α(1,2)-fucosyltransferase synthesizing the H-type 1 antigen in saliva and mucosa, is associated with susceptibility to norovirus infections. Kindberg *et al*. (2006) showed a strong association between the nonsense mutation 428G/A in the *FUT2* gene and slow disease progression of HIV-1 infection. Morrow *et al*. (2011) reported that absent or low expression of salivary H-antigen significantly predicts risk of necrotizing enterocolitis or death in preterm infants.

Johansson *et al*. (2005) reported the interaction of *Helicobacter pylori* with sialylated glycans, preferentially α3-linked; since 3′-SL is found in human milk and bovine colostrum and since its concentration is higher at the start of lactation (Asakuma *et al*., 2007), 3′-SL may be more significant in the prevention of transmission of *H. pylori* from mother to infant than later on. The bovine acidic oligosaccharide fraction, in which 3′-SL is dominant, was shown to reduce by 50% the adhesion of enteropathogenic *E. coli* to Caco-2 cells compared with control cells (Angeloni *et al*., 2005). Terabayashi *et al*. (2006) have shown that it is possible to enhance the inhibition of influenza virus-induced cytopathy elicited by bovine colostrum sialyloligosaccharides by chemical modification; it led to speculation about the future development of anti-infection drugs that utilize bovine colostrum sialyloligosaccharides.

Recently, a pilot study tested the relationship between the consumption of oligosaccharides, oligosaccharide content of feces, and subsequent disease in breast-fed infants and demonstrated that early consumption of HMOs through breastfeeding was also associated with less respiratory and gastrointestinal illness in infants. Although the mechanism by which HMOs prevent respiratory disease in infants is unknown, an immunomodulating effect by a prebiotic mechanism was suggested; alternatively, some of the milk oligosaccharides may have been absorbed into

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the systemic circulation and acted as inhibitory receptor analogs within the respiratory tract (Stepans *et al*., 2006).

Most HMOs cannot be digested within the small intestine and small fractions of HMOs are absorbed intact, perhaps by receptor-mediated endocytosis and could be detected in the urine of breast-fed but not formula-fed infants. There is evidence suggesting that circulating oligosaccharides may have a significant role in the activities of the inflammatory immune response (Rudloff *et al*., 1996). In the review of Mills *et al*. (2011), the process of inflammation has been briefly described and the possible roles of oligosaccharides that harbor epitopes similar to selectin-binding ligands and may play a role in anti-inflammatory processes have been explained. Interestingly, sialyl-Lewis ligands on milk oligosaccharides have been shown to have the greatest capacity for binding selectins *in vitro* (Rudloff *et al*., 2002). Recent studies suggest that oligosaccharides containing sialyl Le^x or its stereoisomer sialyl Le^a, which resemble the P-selectin ligand, inhibit the binding of selectin ligands to the surface of endothelial cells and platelets; this interferes with the formation of platelet–neutrophil complexes, the effect of which is anti-inflammatory (Bode *et al*., 2004). A study by Schumacher *et al*. (2006) identified the acidic fraction of HMOs as effective agents in interfering with P-selectin ligand binding, suggesting that HMOs modulate rather than block the function of P-selectin. It has been reported that the incidence of necrotizing enterocolitis, a condition considered to be an exaggerated immune response, is about 85% lower in breast-fed than in formula-fed infants because of the presence of oligosaccharides. This is consistent with an anti-inflammatory effect of absorbed HMOs (Lucas & Cole, 1990). Eiwegger *et al*. (2004) showed that HMOs affect cytokine production and activation of cord blood-derived T cells *in vitro*. Oligosaccharides, particularly acidic milk oligosaccharides, may therefore influence lymphocyte maturation in breast-fed newborns. The authors concluded that HMOs can modulate the immune system of the maturing infant.

Recently, Daddaoua *et al*. (2006) showed the anti-inflammatory effects of caprine milk oligosaccharides in rats with experimental colitis, suggesting that they may be useful in the treatment of inflammatory bowel disease. A recent study on rats demonstrated that goat milk oligosaccharides reduced the inflammation and body weight loss in a rat model of dextran sodium sulfate-induced colitis (Lara-Villoslada *et al*., 2006).

Sialic acid is found in high concentration in the mammalian nervous system, with the majority being present in gangliosides (65%) and glycoprotein (32%), with the remaining 3% as free sialic acid (Brunngraber *et al*., 1972). Wang and Brand-Miller (2003) suggested that the sialic acid moieties of gangliosides and glycoproteins in the brain frontal cortex play both a structural and functional role and probably participate in a variety of cellular events; gangliosides and glycoproteins have been hypothesized to be involved in the formation of memory and learning. Several studies have demonstrated that the sialic acid concentration in saliva (Wang *et al*., 2001) and in the frontal cortex (Wang, 2009) of breast-fed infants is higher than the level in formula-fed infants. In a study on the nutritional significance of sialic acid in human milk it was demonstrated that dietary sialic acid supplementation during early development enhanced learning and memory in piglets and upregulated the expression of two learning-related genes, UDP-*N*-acetylglucosamine-2-epimerase (*GNE*) and α-2,8-sialyltransferase IV (*ST8SIA4*) (Wang *et al*., 2007). Children who have been breast-fed displayed higher intelligence skills than children who were formula-fed (Smith *et al*., 2003). Some research points out that the sialic acid of sialylated milk oligosaccharides can be absorbed and utilized as a precursor for the biosynthesis of brain gangliosides and sialoglycoprotein and that the feeding of sialylglycoconjugates can improve learning ability (Sakai *et al*., 2006). It may be feasible to use sialyllactose, separated from cheese whey or bovine colostrum, as a functional food for brain activation (Nakamura *et al*., 2003).

A recent study has demonstrated that HMOs have the potential to influence various stages in gastrointestinal development *in vitro* by inducing growth inhibition, differentiation and apoptosis depending on the oligosaccharides (milk fraction or individual oligosaccharides) and human intestinal cell line tested (HT-29, Caco-2, HIECs) (Kuntz *et al*., 2008). Recent methodology based on the use of stable isotope (^{13}C)-labeled oligosaccharides facilitates investigations on their metabolic fate as it allows sensitive determination of the ^{13}C enrichment in biological samples such as feces, blood or urine. ^{13}C-labeled galactose has been used to study how milk oligosaccharides can be absorbed and used as biosynthetic precursors (Rudloff *et al*., 2006). Rudloff *et al*. (2011) reported the renal excretion of intact and partly degraded complex oligosaccharides in 10 infants after *in vivo* labeling of HMOs by administering ^{13}C-Gal to their mothers.

7.6 GENETICS OF CARBOHYDRATE METABOLISM DURING LACTATION

The nutritional and health profile of milk is species-specific and furthermore there are genetic differences within species, due to breeds and selected families. Recent studies indicate that genetic variability could also affect the concentration of milk components with health-promoting effects. Scientists are going beyond the components of milk itself and are examining the genomics of milk – the

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genes that code the composition of milk– with the formation of the Milk Genomics Consortium (German *et al*., 2006), in the expectation that such an approach will reveal the principal biological definition of mammalian nutrition (Mills *et al*., 2011). In recent years a number of papers have been published on glycosylation-related genes and analysis of their expression. In the review of Lepers (2010), a comprehensive analysis of sialyltransferase in vertebrate genomes has been reported. In four mouse tissues about 700 glycan-related enzymes were analyzed with medium-throughput quantitative real-time polymerase chain reaction (qPCR) and microarray approaches (Nairn *et al*., 2008). In humans, Yamamoto *et al*. (2003) described the analysis of 68 glycosyltransferase genes in 27 different tissues by systematic multiplex RT-PCR. García-Vallejo *et al*. (2006) described a reliable method of qPCR to measure the expression levels of 80 human glycosylation-related genes.

The presence of a great variety of oligosaccharides in milk depends on the activity of many different specific enzymes in the lactating gland. As a first step to understanding the complex biology of milk oligosaccharide metabolism, it is important to identify the genes that encode for glycosylation-related enzymes including glycosyltransferases, glycosidases, and sugar transporters. Currently the precise mechanisms regulating differential expression of individual milk oligosaccharides are not known. The production of sialoglycoconjugates is regulated at the level of transcription of glycosyltransferase genes or can be modulated at the cellular level through differential subcellular localization of the enzymes. The identification of genes in animal species and breeds is fundamental for looking at polymorphisms associated with particular oligosaccharide phenotypes that may be objects for selection and other applications.

Many studies have examined gene expression in the mammary gland by qPCR and microarray analysis using biopsy samples. In the mouse mammary gland, Dalziel *et al*. (2001) identified a novel mRNA isoform of the *ST6GAL1* gene containing a novel 5′ untranslated region exon (L) that was associated with a drastic increase in gene expression during lactation. Maksimovick *et al*. (2011) found a similar transcript in rat mammary gland. In contrast, an exon (L)-containing transcript was not detected in the lactating bovine or human mammary gland. They also observed a trend of increasing *ST6GAL1* gene expression in the bovine mammary gland, culminating in involution. This was in contrast to species such as mice where the greatest change in *ST6GAL1* gene expression occurs between pregnancy and lactation, suggesting different roles in rodents compared with other mammals for α2,6-sialylated oligosaccharides present in milk.

A non-invasive sampling procedure starting from somatic cells released into milk during lactation was introduced by Boutinaud and Jammes (2002). Canovas *et al*. (2010) showed that extensive similarities exist between the mammary gland and milk somatic cell transcriptomes. In a recent study by Wickramasinghe *et al*. (2011), the authors compared the milk oligosaccharide profiles at different days of lactation in two bovine breeds with the expression of glycosylation-related genes conducted by RNA sequencing. Ninety-two genes involved in oligosaccharide metabolism were expressed in milk somatic cells and included all sugar transporter, fucose and sialic acid synthesis genes. Selective expression of glycosyltransferase was observed. At all stages of lactation 3′-SL was more abundant than the 6′-SL and, as expected, α2,3-sialyltransferase genes had higher expression levels. The identified candidate gene will be an important start point for the design of targeted breeding strategies to optimize the content of beneficial oligosaccharides in bovine milk.

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8
Milk Bioactive Proteins and Peptides

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8.1 INTRODUCTION

The excellent nutritive value and versatile functional properties of milk proteins are well established. The major protein fractions in milk are caseins and whey proteins which display a number of differences with regard to functional and physiological properties. In comparison to caseins, the whey proteins are biochemically a more diverse group and include α-lactalbumin, β-lactoglobulin, immunoglobulins, lactoferrin, lactoperoxidase, and growth factors (Pihlanto & Korhonen, 2003; Korhonen, 2009a; Pihlanto, 2011a). Most of these proteins are found in abundance in colostrum, reflecting their importance to the growth and development of a newborn offspring (Tripathi & Vashishtha, 2006; Senda *et al*., 2011). All these compounds can nowadays be enriched and purified on an industrial scale from bovine colostral whey or cheese whey (Kitts & Weiler, 2003; Korhonen & Pihlanto, 2007; Jauregi & Welderufael, 2010). Over the last 20 years, scientific research has revealed that milk proteins exhibit a wide range of biological activities that affect digestive functions, metabolic responses to absorbed nutrients, growth and development of organs and disease resistance (Kanwar *et al*., 2009; Korhonen, 2009b; Mills *et al*., 2011). Also, some of these proteins have proven beneficial in reduction of the risks of many chronic human diseases. Recent animal model and human epidemiological and intervention studies suggest that intake of low-fat dairy products or whey protein-based formulations are beneficial in the regulation or prevention of metabolic syndrome, for example obesity, cardiovascular disease, hypertension and type 2 diabetes (Krissansen, 2007; Kris-Etherton *et al*., 2009; Graf *et al*., 2011). Also, a recent intervention study suggested that increase in intake of dairy products attenuated oxidative and inflammatory stress in overweight and obese adults with metabolic syndrome (Stancliffe *et al*., 2011). In animal model and human studies whey proteins have proven better than caseins or soy proteins with regard to weight management but the mechanisms involved remain to be elucidated (Clifton *et al*., 2008; Baer *et al*., 2011; Champagne *et al*., 2011). Recent animal model studies suggest that specific whey proteins or whey protein isolates (WPI) act as active components catalyzing intestinal enzymes and hormones that regulate satiety and digestive processes (Luhovyy *et al*., 2007; Rajic *et al*., 2010; Shi *et al*., 2011). Further animal model and epidemiological studies suggest that milk proteins may also play a role in prevention of certain cancer types, such as colon cancer (Parodi, 2007). These beneficial health effects may be partially, if not fully, attributed to numerous bioactive peptides that are encrypted within intact proteins but which are released during gastrointestinal digestion (Korhonen, 2009c; Mills *et al*., 2011).

This chapter reviews current knowledge about the biological properties of major milk proteins and specific bioactive peptides derived from these proteins. Also, examples of commercial applications of these compounds for human nutrition and health are described. The chapter is devoted to a global milk perspective, even when the scientific literature has so far been overwhelmingly concentrated on cow milk. An overview of bioactive components found in milk of different domesticated mammalian species is given in the recent book by Park (2009) and a review article by Medhammar *et al*. (2012).

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8.2 CASEINS

Cow (*Bos taurus*) milk contains about 3.5% protein, of which the casein fraction constitutes about 80% (28 g/L) and whey proteins 20% (7 g/L) when milk is free of mastitis and low somatic cell count, but even with subclinical mastitis conditions the casein percentage can be markedly reduced. The casein fraction is present mainly in an aggregate colloidal form, termed micelles, which are discrete spherical particles of about 200 nm diameter and which encapsulate insoluble minerals (calcium and phosphate) and small amounts of other minerals (Fox & Brodkorb, 2008). The casein is further divided into α_{s1}-, α_{s2}, β- and κ-casein, which have molecular masses between 19 and 24 kDa and which are assembled to form the complex micelle structure; this comprises α_{s1}-casein and β-casein as the dominant fractions (each about 38–40%) followed by α_{s2}-casein and κ-casein, both representing 10–12% of total casein. These proportions are markedly different for genetic reasons in the milk of many dairy goat breed populations and the milk of other species, especially monogastrics versus ruminants. Current technologies employed in the large-scale fractionation of whole casein from milk are based either on acidic coagulation at an isoelectric point of pH 4.6 or on enzymatic hydrolysis of caseins with rennet. The chemical composition and functional properties of these casein preparations differ from each other. Also, the functional and biological properties of individual casein fractions differ due to differences in their amino acid composition (Akuzawa *et al*., 2009).

Whole casein has nutritional importance primarily because it is the best source of different essential amino acids and is a carrier of calcium and phosphate ions. In addition, whole casein, casein hydrolyzates, and individual casein fractions have been shown, *in vitro* and in animal models, to exert immunomodulatory activities, for example regulating proliferation of lymphocytes and production of antibodies and cytokines (Yousefi *et al*., 2009; Bonomi *et al*., 2011). Moreover, whole casein and individual casein fractions have proven to be a good source of bioactive peptides that can be released by hydrolysis with digestive or microbial enzymes. The production of such peptides has been reported in many articles referring to cow milk (López-Expósito *et al*., 2007; Phelan *et al*., 2009), goat milk (Park, 2009), sheep milk (Recio *et al*., 2009a), and buffalo milk (Pandya & Haenlein, 2009). As these peptides show a wide range of bioactivities, there is now increasing interest in developing methods for industrial isolation of individual casein fractions for inclusion in functional foods and infant formula.

On the other hand, it has been hypothesized that β-casomorphin (BCM)-7, a peptide sequence present in β-casein, is associated with an increased risk for certain non-communicable diseases such as autism, cardiovascular diseases, and type 1 diabetes (McLachlan, 2001). BCM-7 can be released through enzymatic hydrolysis of β-casein but release is determined by the protein's genetic variants, thus being associated with the specific breed. The major polymorphic variants of β-casein in milk of the most common dairy cow breeds are termed A1, A2 and B. The amino acid present at position 67 of the sequence of β-casein is critical for the release of BCM-7. In the A2 variant a proline residue occurs in position 67, whereas the A1 and B variants have a histidine residue in this position. In the case of A2 variant, hydrolysis of the Ile66–Pro67 bond does not occur or occurs at a very low rate. In the case of A1 variant, BCM-7 is released by the action of pepsin. Fresh unprocessed milk does not contain BCM-7 or related peptides. Proteolytic systems involved in fermented milk or cheese manufacture can potentially hydrolyze β-casein to BCM-7 or other BCMs (see review by European Food Safety Authority, 2009).

In human studies, indirect evidence about the release of BCMs from milk during *in vivo* digestion has been reported, but the presence of BCM molecules in blood after intake of milk or casein has not been demonstrated. Some ecological studies have linked BCM-7 intake with cardiovascular disease and insulin-dependent diabetes mellitus in young children, but intervention studies comparing diets containing A1 and A2 variants have not supported such correlations. These studies have been reviewed recently by the European Food Safety Authority, which concluded that a cause–effect relationship between the oral intake of BCM-7 or related peptides and etiology or course of any suggested non-communicable diseases cannot be established (European Food Safety Authority, 2009).

8.3 WHEY PROTEINS

8.3.1 α-Lactalbumin

In cow milk, α-lactalbumin (α-LA) accounts for 2–5% of total protein and about 20% of whey proteins. In colostrum the α-LA content varies from 1.4 to 2.6 g/L and is about 1 g/L in mature milk. The α-LA content in human milk is 3–4 g/L, in buffalo milk 1.4 g/L, in yak milk 2 g/L, in goat milk 1–2.5 g/L, in donkey milk 1.5–2 g/L, in mare milk 3 g/L, in lama milk 3.4 g/L, and in camel milk about 0.9 g/L (Levieux *et al*., 2002; El-Hatmi *et al*., 2006; Pandya & Haenlein, 2009; Medhammar *et al*., 2012; Pecka *et al*., 2012). The molecular mass of cow α-LA is about 14 kDa and it is synthesized in the mammary gland where it acts as a coenzyme for lactose biosynthesis. In human milk α-LA is the predominant whey protein, constituting about 10–20% of total protein in mature milk. α-LA from cow milk and its hydrolyzates can be used as supplements in

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infant formula owing to the high degree (74%) of amino acid homology to human α-LA (Lönnerdal, 2011) and a few α-LA enriched formulas have been commercialized. However, intact α-LA is one of the major allergens in cow milk. Binding of calcium influences spatial conformation and stability of α-LA which also functions as a calcium carrier. Furthermore, α-LA is a good source of many bioactive peptides, such as angiotensin-converting enzyme (ACE)-I inhibitor, and opioid and antimicrobial peptides (Pihlanto, 2011b).

α-LA may exert many biological functions. Because of its high tryptophan content (5%), α-LA may have some effect on serotonin metabolism. An α-LA-based tryptophan-rich supplement was reported to improve cognitive functions in stress-vulnerable subjects (Markus *et al*., 2002). α-LA is reported to have gastric epithelia protecting and anti-inflammatory activity in rat models, for example against experimentally induced gastric mucosal injury caused by intake of ethanol or non-steroidal anti-inflammatory drugs (NSAIDs) (Rosaneli *et al*., 2002, 2004).

A particular conformational form of human and also cow α-LA induces apoptosis in tumor and immature cells while sparing healthy differentiated cells. The conversion of α-LA to the apoptotic form requires unfolding of α-LA and binding of specific fatty acids, mainly unsaturated C18 fatty acids in the *cis* conformation (Rammer *et al*., 2010; Barbana *et al*., 2011). Purified porcine, equine and caprine α-LA forms also complex with oleic acid and express similar apoptotic activities (Pettersson *et al*., 2006). Thus, α-LA in the milk of several species could have preventive effects on gastrointestinal cancer similar to that found earlier for human α-LA. α-LA is rich in the sulfur amino acid cysteine (6%) and it has been demonstrated that intake of a cysteine-rich whey protein diet tends to improve glycemic control and alleviate sucrose-induced oxidative stress and development of insulin resistance in rats fed a high-sucrose diet (Blouet *et al*., 2007). In a mouse model, a high-calcium diet (1.8% $CaCO_3$) with α-LA (18% of energy) significantly increased weight loss during an energy restriction diet. When the mice were subsequently fed a high-fat diet *ad libitum*, the α-LA reduced the weight regain and fat accumulation as compared with WPI (Pilvi *et al*., 2009). These animal studies are encouraging in view of the potential use of α-LA in dietary regimes that try to reduce the risk of developing obesity, metabolic syndrome, and type 2 diabetes. To this end, intervention trials in humans are required.

8.3.2 β-Lactoglobulin

β-Lactoglobulin (β-LG), an 18.3-kDa protein, is one of the main proteins in cow milk whey (3.0–3.5 g/L), accounting for about 50% of the whey proteins. β-LG is also present in buffalo, mare, donkey, yak, and goat milks but is not found in human and camel milk (Chatterton *et al*., 2006). It occurs as a reversible dimer at the native pH of milk and binds a range of small hydrophobic and amphipathic molecules, such as fatty acids, retinol, and vitamin D_2. β-LG can be isolated on an industrial scale from cheese whey using chromatographic and membrane separation techniques. β-LG exhibits many functional and nutritional characteristics that have made this protein a multifunctional ingredient for many food and biochemical applications. It has excellent heat-set gelation properties and is therefore used in products which require good water-binding and texturizing properties. Regarding biological functions, β-LG has been associated with antimicrobial, anticarcinogenic and hypocholesterolemic effects and with prevention of pathogen adhesion (Chatterton *et al*., 2006). Owing to its lipophilic property, it has been suggested that this whey protein may play a role in the absorption and subsequent metabolism of specific fatty acids. Further, β-LG has been applied in experimental studies to show the importance of lipid–protein interactions for gastrointestinal proteolysis of specific proteins (Mackie & Macierzanka, 2010). Such colloidal interactions may play a role in protein and fat metabolism and could therefore be exploited in optimizing nutrition. Furthermore, β-LG from cow, goat and sheep milk has proven to be an excellent source of peptides with a wide range of bioactivities, such as antihypertensive, antimicrobial, antioxidative, anticarcinogenic, immunomodulatory, opioid, hypocholesterolemic, and other metabolic effects (Hernández-Ledesma *et al*., 2008; Park, 2009; Recio *et al*., 2009a). Recently, it has been observed that glycation of cow milk β-LG with galactooligosaccharides (GOS) via the Maillard reaction forms stable glycated peptides in subsequent simulated digestion. These glycated peptides seem to express similar bifidogenic activity compared with unconjugated GOS (Hernández-Hernández *et al*., 2011). This finding could open up new applications for Maillard reaction products as prebiotic compounds.

8.3.3 Glycomacropeptide

Glycomacropeptide (GMP) is a C-terminal glycopeptide f(106–169) released from the κ-casein molecule by the action of chymosin. GMP is hydrophilic and remains in the whey fraction in the cheese manufacturing process. GMP is the most abundant protein fraction in cheese whey, amounting to 20–25% of the proteins (Abd El-Salam *et al*., 1996). GMP contains a significant carbohydrate fraction (50–60% of total GMP) that is composed of galactose, *N*-acetylgalactosamine, and *N*-neuraminic acid. The non-glycosylated form of GMP is termed caseinomacropeptide or CMP. Pure GMP can be recovered in large quantities

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from cheese whey by chromatographic or ultrafiltration techniques (Thomä-Worringer *et al*., 2006). It has been suggested that GMP has many biological properties but clinical evidence about potential health benefits is still rather limited. In *in vitro* studies GMP has been shown to inactivate microbial toxins of *Escherichia coli* and *Vibrio cholerae*, inhibit adhesion of cariogenic bacteria and influenza virus, modulate immune system responses, promote growth of bifidobacteria, suppress gastric hormone activities, and regulate blood circulation through antihypertensive and antithrombotic activity (Manso & López-Fandino, 2004). Also, CMP from milk of several animal species has been reported as a good source of antithrombotic peptides. A recent *in vitro* study (Setarehnejad *et al*., 2010) has suggested that CMP and its fractions can provide a protective effect against dental erosion when applied, for example, in acidic drinks.

Owing to its glycoprotein nature, GMP has interesting nutritional and physicochemical properties. GMP is rich in branched-chain amino acids and low in methionine, which makes it a useful ingredient in diets for patients suffering from hepatic diseases. GMP contains no phenylalanine and is therefore suitable for patients suffering from phenylketonuria. Animal model studies have suggested that the high sialic acid content of GMP may deliver beneficial effects for brain development and improvement in learning ability (Wang *et al*., 2007). Furthermore, GMP is speculated to regulate appetite and this potential effect has been investigated in a number of studies, as reviewed by Recio *et al*. (2009b). Animal model and *in vitro* studies have shown that GMP inhibits gastric secretions and reduces stomach motility by stimulating the release of cholecystokinin (Burton-Freeman, 2008; Guilloteau *et al*., 2010). This hormone is involved in controlling food intake and digestion in the duodenum of animals and humans. An animal model study (Royle *et al*., 2008) demonstrated that both GMP and a WPI decreased weight gain and altered body composition in male Wistar rats. GMP had a significant additional effect on reduction of fat accumulation when combined with WPI, but the mechanism of this effect was not clear. On the other hand, GMP may have a beneficial role in modulation of gut microflora, as this macropeptide has been shown to promote the growth of bifidobacteria (Recio *et al*., 2009b). There is some indication from mouse model and human studies that bifidobacteria residing in the gut may affect weight gain. Further research in human subjects is needed to confirm these findings. Commercial products and supplements containing GMP are now available in many countries for the purpose of appetite control and weight management. However, the efficacy of these products remains to be established in clinical studies.

8.3.4 Lactoferrin

Lactoferrin (LF) is an iron-binding glycoprotein found in colostrum, milk, other body fluids, and in neutrophilic leukocytes. Experimental and clinical research carried out over the last 30 years has accumulated much evidence about the beneficial health effects of LF and its derivatives. LF is an important host defense molecule exhibiting a diverse range of physiological functions. At present, the major known *in vivo* activities of cow milk LF and peptides derived thereof include defense of the udder and gastrointestinal tract against microbial pathogens, sequestering and transport of iron, regulating immune system activities, antineoplastic activity, and mitogenic and trophic activities in the gastrointestinal tract and associated lymphoid tissue (GALT) (Marnila & Korhonen, 2009).

LF is synthesized in the epithelial cells of the mammary gland, but is present at high concentrations also in neutrophils. In cow colostrum, the LF content is on average 1.5 g/L (range 0.2–5 g/L) and decreases to around 0.1 g/L (range 0.07–1.2 g/L) in mature milk. The LF content of human milk is 0.7–1.5 g/L, of buffalo milk 0.32 g/L, of yak milk 0.67 g/L, of donkey milk 0.3 g/L, and of camel milk 0.2–0.3 g/L (El-Hatmi *et al*., 2006; Konuspayeva *et al*., 2007; Pandya & Haenlein, 2009; Medhammar *et al*., 2012). LF is delivered by degranulating neutrophils into sites of microbial invasion, as in mastitic milk in which the LF content is manifold.

Cow LF is composed of a single-chain polypeptide sequence (689 amino acids, 69% sequence homology with human). Molecular mass is 80–84 kDa depending on the degree of glycosylation. Each LF molecule can bind with high affinity (K_d ~10^{-30}) two ferric ions (Fe^{3+}) with concomitant incorporation of bicarbonate ion (HCO_3^-) or carbonate ion (CO_3^{2-}). The iron saturation rate of cow milk LF is 20–30%. Iron-depleted LF (< 5% saturation) is called apo-LF whereas the saturated form is referred to as holo-LF. Under physiological conditions (pH 7–8), LF is positively charged and strongly binds polyanions such as heparin, bacterial lipopolysaccharide (LPS), lysozyme, immunoglobulins (especially IgA), casein, and DNA.

The structure of LF, its antibacterial activity and bacterial interaction are not markedly affected by the standard pasteurization regimes (72 °C for 15 s) used in the dairy industry. However, UHT treatment abolishes the ability of holo-LF to bind to bacteria as well as the bacteriostatic activity of apo-LF. In spray-drying of milk, a marginal loss of LF activity is observed. In heat treatments, apo-LF denatures faster than holo-LF (Marnila & Korhonen, 2009).

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8.3.4.1 Antimicrobial effects

The *in vitro* antimicrobial activities of LF or its cleavage products are well documented (Wakabayashi *et al*., 2006; Weinberg, 2007; Jenssen & Hancock, 2009). The effects are exerted by several different mechanisms, which can be divided into the following main patterns:

- iron sequestering in order to deprive bacteria of iron;
- binding to bacterial membrane structures leading to disruption of cellular metabolism;
- protease-like antimicrobial activity toward some bacterial virulence factors;
- induction of apoptosis in host cells infected by some intracellular pathogenic bacteria;
- inhibiting viral replication by preventing viruses from infecting new host cells;
- antifungal activities by binding to fungal cell walls and by upregulating neutrophil, natural killer and killer cell functions.

The apo-LF can inhibit the growth and decrease the virulence of a variety of pathogenic bacteria and yeasts, such as enteropathogenic *E. coli* (EPEC), *Helicobacter pylori*, *Klebsiella*, *Salmonella*, *Proteus*, *Pseudomonas*, *Listeria*, *Bacillus*, *Streptococcus* and *Candida albicans*. The transition of bacteria from a free-living form into biofilms can be devastating for the host, because biofilms protect bacteria effectively from host defense mechanisms and antibiotics. Chelation of iron by LF can in many cases block biofilm development of bacteria. The iron deprivation-related bacteriostatic effect is most pronounced with respect to EPEC or antibiotic-resistant bacterial strains (Yen *et al*., 2011). There is some variation in the antimicrobial efficacies of LFs from milks of different species. LF isolated from the Bactrian camel (*Camelus bactrianus*) milk had, after 24 hours of incubation, over four times lower minimum inhibitory concentration against *E. coli* O157:H7 than LF from human or alpaca (*Lama pacos*) milk (Conesa *et al*., 2008). Clinical trials show evidence that use of cow milk LF provides a means to prevent sepsis and necrotizing enterocolitis in preterm neonates (Manzoni *et al*., 2011; Pammi & Abrams, 2011).

The presence of apo-LF enhances the efficacy of many other antimicrobial agents, since the function of the majority of antimicrobial compounds is inhibited by iron. Lysozyme (LZM) alone is not considered to be very effective against Gram-negative bacteria but in combination with LF it is far more bactericidal against both Gram-negative and Gram-positive bacteria than LF or LZM alone (Leitch & Willcox, 1998). Coincubation of *Staphylococcus aureus* with β-LG and LF resulted in augmented antibacterial activity, suggesting synergistic activity of the proteins (Chaneton *et al*., 2011). Cow LF added to penicillin offered an effective combination for the treatment of cows suffering from mastitis caused by β-lactam antibiotic-resistant *S. aureus* strains (Komine *et al*., 2006).

LF inhibits the replication of a wide spectrum of RNA and DNA viruses, including several enveloped viruses like herpes simplex viruses, hepatitis B and C viruses, human cytomegalovirus, hantavirus, HIV, respiratory syncytial virus, and also some naked viruses such as rotavirus, poliovirus, adenoviruses, and human papillomavirus. LF inhibits viral replication by preventing the viral particles from infecting new host cells either by binding to host cell receptors or to the virions. The observation that LF is strongly antiviral when present at the time of infection but far less active if given later suggests that antiviral therapy with LF may not be practicable in many cases (Berlutti *et al*., 2011).

Cow milk LF and peptides derived from it have bactericidal activities *in vitro* against pathogenic fungi, especially *Candida* species, including *C. albicans*. The *in vitro* effect is related to the adsorption of LF to the cell surface rather than to iron deprivation. Clinical and animal model studies suggest that the main antimycotic activity *in vivo* is exerted by enhancing host immune defense, especially neutrophil function, rather than by a direct bactericidal effect (Ueta *et al*., 2011). Orally administered cow milk LF was effective in treatment of moderate vesicular or interdigital tinea pedis (Yamauchi *et al*., 2000) and a topically applied cream containing LF (4%) cured completely most of the patients suffering from acute vulvovaginal candidiosis (Costantino & Guaraldi, 2008). Thus, the use of LF as an antifungal agent alone or in combination with antifungal drugs opens promising prospects for the therapy of opportunistic fungal infections.

8.3.4.2 Immunological effects and cancer prevention

Several cell types of the immune system, including T and B lymphocytes, platelets and intestinal epithelial cells, have receptors for LF (Suzuki *et al*., 2005; Lönnerdal *et al*., 2011). It is therefore likely that orally administered cow milk LF or its digestion products act on the immune system in the GALT and also promote systemic effects. LF has both immune system-stimulating and inflammatory cascade-inhibiting effects. A daily intake of 100 mg of LF orally for 7 days followed by 200 mg for 7 days resulted in statistically significant increases in total T-cell activation, helper T-cell activation, and cytotoxic T-cell activation in healthy males (Mulder *et al*., 2008).

LF can control the cytokine-induced proinflammatory cascade during the development of a systemic inflammatory response in tissues (Kruzel *et al*., 2007). LF augments chemotactic activity of neutrophils, favoring their rapid

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recruitment from blood to inflammatory sites. Activation of the phagocyte system results in the production of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, which in turn enhances the inflammatory reaction. LF can attenuate the priming reaction and onset of production of proinflammatory cytokines via the nuclear factor (NF)- κB and by stimulating the production of the anti-inflammatory cytokines IL-4 and IL-10. By scavenging free iron, apo-LF can reduce leukocyte-mediated oxidative damage in inflamed tissues. In the case of an infection by Gram-negative bacteria, LF binds to LPS and inhibits the progressive inflammatory cascade caused by cell wall particles of dead bacteria (Kruzel *et al*., 2007). LF appears to be an essential part of the regulatory feedback loop in the inflammatory process.

LF has been shown to be transported into the cell nucleus where it can bind DNA, suggesting that it may regulate the phenotypic traits of the host. Animal model studies suggest that LF and peptides derived from it participate in the defense against development and progression of tumors. LF from cow milk had a synergistic effect with black tea polyphenols in the prevention of chemically induced genotoxicity and development of carcinomas in hamster (Mohan *et al*., 2008). Thus far, relatively little is known about the mechanisms by which LF exerts its anticancer activity. Lactoferricin is *in vitro* a potent inducer of apoptosis in several different human leukemia and carcinoma cell lines (Mader *et al*., 2005). The antitumor effect may be due to inhibition of angiogenesis and by an activating effect on cell-mediated cytotoxicity reactions against neoplasia. LF has been demonstrated to activate natural killer cells, polymorphonuclear leukocytes, and lymphokine-activated killer cells (Kruzel *et al*., 2007).

8.3.4.3 Applications and safety aspects

LF from cow milk and recombinant human LF are now commercially available for development as preservatives, nutraceuticals, and pharmaceutical products. The expanding knowledge about the bioactivities of LF has enlarged its scope of applications, from food preservation to health-promoting supplements, infant milk formula, fish feeds, pharmaceuticals, healthcare, oral hygiene products, and cosmetics. Current research is focused on bone remodeling (Malet *et al*., 2011), prevention of infections, prevention of neoplasia and treatment of cancers, treatment of inflammation in the intestine, and anemia (Wakabayashi *et al*., 2006; Weinberg, 2007; Tomita *et al*., 2009).

Orally administered 30% iron-saturated cow milk LF can influence iron homeostasis directly or through other proteins of intestinal cells. LF increased the hemoglobin and serum total iron concentration in women suffering from iron-deficiency anemia. Unlike ferrous sulfate, LF did not result in any side effects (Paesano *et al*., 2010).

The results from toxicological studies suggest that cow milk LF can be regarded as a safe ingredient for use in food products and supplements (Tamano *et al*., 2008). The US Food and Drug Administration (FDA) has approved the use of bovine LF (at not more than 2% by weight) as a spray to reduce microbial contamination on the surface of raw beef carcasses. The FDA has granted to bovine LF a "Generally Recognized As Safe" (GRAS, GRN 67) status and this determination accounts for uses at defined levels in beef carcasses, sub-primals, and finished cuts (Taylor *et al*., 2004). However, many pathogenic strains of *E. coli* and *S. aureus* show resistance or can develop resistance to LF. Thus, widespread use of LF or lactoferricin in, for example, the food industry may lead to development of resistant pathogens and in the case of LF or lactoferricin the potential pathogens would in practice resist the molecules of human innate immunity. Immunological effects of orally administered LF are diverse and only partially known. Therefore, further experimental research concomitant with relevant safety evaluation appears necessary in those particular application areas.

8.3.5 Lactoperoxidase and lysozyme

8.3.5.1 Lactoperoxidase

Lactoperoxidase (LP) (EC 1.11.1.7) is a peroxidase with broad substrate specificity. LP is chemically a glycoprotein and has a molecular mass of about 78 kDa. It occurs naturally in colostrum, milk, saliva and many other human and animal secretions. LP represents the most abundant enzyme in cow milk (about 30 mg/L) and can be recovered in substantial quantities from whey using chromatographic techniques. LP from cow milk is relatively heat-resistant, retaining about 50% of its original activity after high-temperaturee short time (HTST) pasteurization (Kussendrager & van Hooijdonk, 2000).

LP catalyzes an antimicrobial system (LP system) consisting of the thiocyanate anion (SCN^-) and hydrogen peroxide (H_2O_2) to generate short-lived oxidation products, primarily hypothiocyanate ($OSCN^-$) (Reiter & Perraudin, 1991). These highly reactive compounds cause oxidation of sulfhydryl groups in microbial enzymes and other membrane proteins, killing or inhibiting the growth of a wide range of microorganisms, including bacteria, viruses, fungi, molds, and protozoa (Seifu *et al*., 2005). With Gram-positive bacteria, for example *Listeria* spp., *Staphylococcus* spp., and *Streptococcus* spp., the inhibition is bacteriostatic, while Gram-negative bacteria such as *E. coli*, *Salmonella* spp., *Pseudomonas* spp., *Campylobacter* spp., and *H. pylori* are killed. Nowadays, the LP system is considered to be an important part of the natural host defense system in mammals and the protective function seems to be mediated

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by several mechanisms (Boots & Floris, 2006). LP is resistant to low pH (as low as 3) and to human gastric juice. The thiocyanate ion is widely distributed in animal secretions and tissues. Hydrogen peroxide may be formed endogenously, as many lactobacilli, lactococci and streptococci produce sufficient amounts of H_2O_2 under aerobic conditions to activate the LP system. H_2O_2 can also be produced *in situ*, for example by adding glucose oxidase and glucose to the medium (Reiter & Perraudin, 1991).

In animal studies, the LP system has been shown to reduce diarrhea in newborn calves (Reiter & Perraudin, 1991) but similar studies in humans have not been reported. On the other hand, the LP system is known to contribute to the natural defense mechanisms in human saliva against dental caries (Ihalin *et al*., 2006). This antibacterial system is particularly effective against the cariogenic bacterium *Streptococcus mutans* and periodontitis-associated bacteria such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Also, the LP system has been shown to prevent the *in vitro* growth of *H. pylori* (Shin *et al*., 2002) and bacterial colonization of the airway epithelium (Gerson *et al*., 2000). The LP system is known to provide a synergistic antimicrobial action with other natural defense components, such as LF, lysozyme and antibodies (Floris *et al*., 2003). This synergistic action may take place, for example, in saliva and milk where all necessary components can be available. Oral administration of LP in combination with LF has been demonstrated to attenuate symptoms of pneumonia in influenza virus-infected mice through suppression of infiltration of inflammatory cells in the lung (Shin *et al*., 2005). Further, this system has been shown to be inhibitory to *Candida* spp. and the protozoan *Plasmodium falciparum* and it can inactivate *in vitro* HIV-1 and poliovirus (Seifu *et al*., 2005). The overall physiological significance of the LP system still remains to be elucidated.

The best-studied antimicrobial function of the LP system is related to its ability to preserve raw milk of different domestic animals, such as cow, buffalo, camel, and goat (see reviews by FAO/WHO, 2006 and Pandya & Haenlein, 2009). Raw milk contains all necessary components to make the system functional but the natural concentrations in milk of thiocyanate and H_2O_2 in particular are often below the required level. Therefore, activation of this antibacterial system usually requires supplementation of raw milk with both these components. The effectiveness of the activated LP system in milk of different species (e.g., cow, buffalo, camel and goat) has been demonstrated in many field trials worldwide (Seifu *et al*., 2005; FAO/WHO, 2006; Ponce, 2010). The LP system was approved in 1991 by the Codex Alimentarius Committee for preservation of raw milk under conditions where facilities for milk cooling are insufficient. The safety of the LP system was established by the same committee and this statement was confirmed by an FAO/WHO technical meeting in 2005 (FAO/WHO, 2006). Since 1991, the LP system has being applied in a number of developing countries. The LP system has also found applications in dental healthcare products and animal feeds. Recent novel potential applications include preservation of easily perishable products, for example meat, fish, vegetables, fruits, and flowers (Boots & Floris, 2006).

8.3.5.2 Lysozyme

Lysozyme (LZM), also known as muramidase (peptidoglycan *N*-acetylmuramoyl hydrolase; EC 3.2.1.17), is a potent antibacterial enzyme (of about 15 kDa) that catalyzes the hydrolysis of the β-$(1\rightarrow4)$ linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine of bacterial peptidoglycan (Floris *et al*., 2003). This structural component is particularly abundant in the cell wall of Gram-positive bacteria. LZM is widely distributed in various biological fluids and tissues, including avian egg, plants, bacteria, and animal secretions, but its concentration varies considerably in different sources. Hen egg white (1–3 g/L), human milk (up to 0.4 g/L), mare's milk (0.4–1 g/L) and donkey's milk (1–2 g/L), are abundant sources of LZM. Also, tears and saliva contain significant amounts of LZM whereas its concentration in cow and buffalo colostrum and milk is relatively low (0.4 mg/L) (Floris *et al*., 2003; Pandya & Haenlein, 2009).

Since the discovery of LZM by Alexander Fleming in the early 1920s, LZM has been known as an effective antimicrobial agent. Lysozymes present in different sources vary with regard to their antibacterial spectrum and specificity toward different types of mucopolysaccharides. LZM shows high activity against mesophilic and thermophilic spore-forming bacteria, such as bacilli and clostridia, but is also active against other spoilage and pathogenic microorganisms, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Candida albicans*, and *Streptococcus mutans* (Johnson, 1994). LZM, LF, and antibodies have a synergistic bactericidal effect on many microorganisms (Pan *et al*., 2007).

In addition to antibacterial activity, LZM has many other functions, for example inactivation of certain viruses, enhancement of antibiotic effects, anti-inflammatory and antihistamine actions, activation of immune cells and antitumor activity (Floris *et al*., 2003).

LZM can be effective even after oral administration, since it is rather resistant to digestive enzymes (Sava, 1996). Oral and topical applications of LZM have been found to be effective in preventing and controlling several viral skin infections, including herpes simplex and

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chickenpox (Sava 1996). In food applications, egg white LZM is being utilized as a preservative in fermented cheese varieties to prevent the late fermentation defect caused by contaminating butyric acid bacteria. The potential physiological significance of natural milk LZM has not been elucidated fully.

8.3.6 Growth factors and cytokines

Cytokines, hormones and specifically named growth factors are all signaling molecules released by cells to communicate with each other. They are all important for the regulation, growth, maturation or repair of different cell types. In general, the growth factor concentration is the highest in colostrum during the first hours of calving and decline substantially after that (Gauthier *et al*., 2006a). These non-nutrient components contribute to the specific stimulation of jejunal and skeletal muscle development in colostrum fed neonates.

Epidermal growth factor (EGF) and β-cellulin (BTC) belong to the EGF family. In colostrum and mature milk from cow the concentrations of EGF vary between 4–320 μg/L and 2–155 μg/L, respectively. They are sufficient to induce physiological responses. BTC is present in cow colostrum (2.30 μg/L) and in cheese whey (2.59 μg/L). Members of the EGF family stimulate the growth of epithelial cells, the predominant function being to be readily available to stimulate repair at sites of gastrointestinal damage. EGF inhibits the secretion of gastric acid and modulates the synthesis of a number of hormones (Ghosh & Playford, 2006).

Among the transforming growth factor (TGF)-β family, TGF-β2 is the predominant isoform, present at high concentrations in cow colostrum and milk (150–1150 and 13–71 μg/L, respectively). Also TGF-β1 is detected in both cow colostrum and milk (12–43 and 0.8–4 μg/L, respectively). Members of the TGF-β family are suggested to be mucosal integrity peptides, maintaining normal epithelial function in the non-damaged mucosa (Gauthier *et al*., 2006a; Ghosh & Playford, 2006).

Insulin-like growth factor (IGF)-I and IGF-II promote cell proliferation and differentiation and have anabolic activities. In milk they are thought to contribute to growth and development of the neonate. In milk, the IGFs are found almost entirely bound to high-affinity binding proteins. The IGF peptide is released from binding proteins by acid treatment. Colostrum from cow contains 32–800 μg/L of IGF-I and mature milk 4–27 μg/L (Gauthier *et al*., 2006a; Ghosh & Playford, 2006).

Platelet-derived growth factor (PDGF) is an acid-stable molecule synthesized and secreted by platelets and macrophages. PDGF is a potent mitogen for fibroblasts and facilitates ulcer healing when administered orally to animals. Although PDGF is found in milk it has not been quantified (Gauthier *et al*., 2006a).

Fibroblast growth factor (FGF)-I and FGF-II can exert multiple functions on a variety of cells playing an important role in proliferation, differentiation and survival of a variety of different cell types. FGF-II has potent angiogenic properties and is involved in wound healing and hematopoiesis. FGF-I is detected in cow milk at a concentration of 6 ng/L, whereas FGF-II is detected at 20 ng/L in milk and 0.5–1 μg/L in colostrum (Gauthier *et al*., 2006a).

Molecules referred to as cytokines have a broad range of cellular functions. In general, cytokines do not regulate normal cellular homeostasis but act to alter cellular metabolism and to trigger acute cellular responses as during inflammation (Ghosh & Playford, 2006). Bovine colostrum and mastitic milk contain a number of cytokines derived from leukocytes, epithelial cells or lymphoid tissues of the udder. These cytokines, such as IL-1β, TNF-α, interferon-γ and soluble IL-1 receptor agonist, are important messengers within the immune system. These are also important in stimulation of the calf's immune system.

Osteopontin (OPN) is a pleiotropic cytokine secreted by macrophages, T cells and epithelial cells and is known to induce cell-mediated immune responses, chemotaxis and also anti-inflammatory responses. A secreted form of OPN is involved in generation of T helper type 1 (Th1) and Th17 cells that are pathogenic for various autoimmune diseases. OPN is highly expressed at the pathological foci of various inflammatory diseases (Uede, 2011) and in high concentrations in milk (Lönnerdal, 2011) but its function in milk is not clear. A significant proportion of milk OPN is expected to pass through the gut and into the intestines largely intact on milk consumption since the protein is relatively resistant to proteolysis by the neonatal gastric juice. This suggests that OPN or its fragments could regulate the infant immune response. OPN has been shown to form a strong electrostatic complex with LF, each OPN molecule being capable of binding three LF molecules. OPN may protect LF from proteolysis and aid its transport to specific sites in the intestinal mucosa (Lönnerdal, 2011).

In recent years, pilot or semi-industrial scale methods have been developed for extraction of growth factors from cow colostrum and cheese whey. An acid casein extract rich in TGF-β2 has been tested successfully in children suffering from Crohn's disease and another growth factor extract from cheese whey has shown promising results in treatment of oral mucositis and wound healing both in test animal trials and in humans (Smithers, 2008). Other potential applications could be treatment of psoriasis and allergies, and cytoprotection against intestinal damage caused by chemotherapy. Cheese whey and colostrum-based products have found growing

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worldwide markets as dietary supplements. Also, colostrum-based products claimed to improve sports performance are commercially available. However, the scientific clinical evidence related to these supplements remains disputed (Shing *et al*., 2009).

8.3.7 Immunoglobulins

The function of immunoglobulins in colostrum and milk is to provide the calf with immunological protection against environmental pathogens and toxins during the development of its own immune system and to protect the udder against infections. In ruminants the placenta does not allow significant transfer of macromolecules and the immunoglobulins are absorbed from the colostrum into blood circulation of a newborn calf within the first 24–36 hours after birth. Probably due to this unique function, immunoglobulins account for 70–80% of total protein in cow colostrum whereas in milk they account for only 1–2% of total protein content. The concentration of immunoglobulins in cow milk varies according to breed, age, health status, and stage of lactation. In the first colostrum the IgG content is 20–180 g/L and declines rapidly to 0.3–1 g/L in mature milk. The reported IgG content is essentially similar in goat, camel, donkey, mare, and yak milk (Levieux *et al*., 2002; Konuspayeva *et al*., 2007; Medhammar *et al*., 2012; Pecka *et al*., 2012). The immunoglobulin classes present in bovine milk are IgG1, IgG2, IgM and IgA, the major class being IgG1 (Korhonen & Marnila, 2009). The properties of immunoglobulin classes are presented in Table 8.1.

8.3.7.1 Functions of immunoglobulins

All immunoglobulin classes function as flexible and versatile adaptors linking various parts of the cellular and humoral immune system by binding to antigens and exhibiting one or more effector functions. The Fab part of the immunoglobulin molecule binds to antigen and other parts (mostly the Fc region) interact with other elements such as the leukocyte receptor. The monomeric IgG class antibodies possess a multitude of immune functions including opsonization, complement fixation, agglutination of bacteria, prevention of bacterial adhesion to endothelial cell lining, inhibition of bacterial metabolism by blocking enzymes, and neutralization of viruses and toxins (Mehra *et al*., 2006). Pentameric IgM antibodies are considerably more effective in most of the aforementioned activities than IgG, especially in complement fixation. Secretory (S)IgA does not fixate complement but is effective in neutralizing viruses and toxins and in agglutinating bacteria.

Preventing adhesion of pathogenic microbes to surfaces like epithelial cell linings or mucous membranes may be the most important mechanism of milk immunoglobulins in protecting the host. The attachment is for many microbes the critical first step in the establishment of colonization and infection (Korhonen & Marnila, 2009). The agglutinating ability of immunoglobulins reduces the ability of bacteria to adhere on surfaces. Colostrum and milk are known to contain agglutinating immunoglobulins for a large variety of microorganisms. Immunoglobulins can inhibit bacterial metabolism and reduce the production of harmful components such as toxins by blocking

Table 8.1. Immunoglobulins in cow colostrum and milk: concentrations and physicochemical and immunological properties.

	IgG1	IgG2	IgA	SIgA	IgM
Concentration (g/L)					
Colostrum*	60 (15–180)	1–3	Traces	3.5 (1–6)	5 (3–9)
Milk	0.35 (0.3–0.6)	0.02–0.12	Traces	0.05–0.14	0.04–1.0
Physicochemical properties					
Mass (kDA)	146–163	146–154	160	385–430	900
Structure	Monomer	Monomer	Monomer	Dimer	Pentamer
Carbohydrates (%)	2.8–3.1	2.6–3.0	6–10		10–12
Immunological functionality					
Opsonization	+++	+	0	0	+++
Complement fixation	++	+	0	0	+++
Agglutination	+	+	++	++	+++

*First milking post partum.

0, no activity; +, low activity; ++, moderate activity; +++, strong activity.

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receptors or enzymes on bacterial surfaces. This blocking may inhibit the ability of pathogens to produce structures needed in adherence to surfaces.

Immunoglobulins bind and neutralize toxins. Many bacterial toxins must first be transported via receptors inside the host cells to cause cell death and binding to immunoglobulin may prevent the internalization. Microfold cells in the intestinal epithelial lining have specific receptors for SIgA and are able to bind and transcytose antigen–SIgA–receptor complexes into aggregated lymphoid nodules (Peyer's patches) for antigen presentation and induction of mucosal immune responses.

Specific immunoglobulins augment the recognition and phagocytosis of microbes by phagocytic leukocytes (opsonization). Leukocytes are an integral part of normal milk and colostrum. Immunoglobulins may contribute to opsonization and killing of pathogenic microbes by also activating the milk complement system. However, its significance in milk may be limited in immune defense of the udder. The milk immunoglobulins also have synergistic effects with some non-specific antimicrobial factors in milk, such as LZM, LF and the LP–thiocyanate–H_2O_2 system (Loimaranta *et al*., 1998; Bostwick *et al*., 2000).

8.3.7.2 Immunoglobulins and immune milk preparations

The development of industrial-scale fractionation technologies has raised interest in developing products with supplemented immunoglobulins. Most of the current immunoglobulin products are prepared from colostrum of cheese whey by removing the fat, followed by microfiltration or pasteurization under conditions that retain the biological activity of immunoglobulins. Heating at 60 °C for 120 min does not affect the neutralizing activity of IgG, but at temperatures above 65 °C the biological activities can be lost rapidly (McMartin *et al*., 2006). Immunoglobulin products are typically in the form of freeze-dried or spray-dried powders, the immunoglobulin content being 30–70% of dry weight. Some products are in the form of filtered colostral whey liquids or concentrates. The low pH in the stomach significantly reduces the bioactivities of ingested immunoglobulins and furthermore the immunoglobulins are subjected to degradation by intestinal proteases. However, the secretory piece of SIgA protects it from proteolytic enzymes. The Fab and $F(ab')_2$ fragments resulting from degradation of IgG retain part of the neutralizing and adhesion-inhibiting activities of immunoglobulins in the intestine (Pacyna *et al*., 2001). Encapsulation of immunoglobulins with gelatine increases remarkably the survival of immunoglobulins.

Colostral immunoglobulin preparations designed for farm animals have been commercially available for a long time. Cow colostrum-based immunoglobulin concentrates for humans have also found a growing worldwide market as dietary supplements (Tripathi & Vashishtha, 2006; Struff & Sprotte, 2007). These are advertised as health-promoting supplements boosting natural immunity and maintaining general well-being without any specific microbial target. Some of these products have been tested clinically for certain physiological efficacies, such as improving recovery from strenuous exercise, for boosting specific immune functions or for prevention or treatment of microbial infections. In most cases the clinical evidence related to these products is very limited or remains disputed.

On the other hand, the oral administration of immune or "hyperimmune" preparations has resulted in promising results in prevention of many microbial infections in humans. Immune preparations are made by raising specific antibodies in cow colostrum by a systematic immunization protocol of the animal before parturition (Korhonen & Marnila, 2009). By this means the specific antibody titer against certain pathogenic microbes or virulence factors can be raised up to several hundred times. The resulting immune colostra or preparations made thereof have fundamentally different efficacies than normal colostrum or antibody concentrates from normal milk. For this reason, the immunoglobulin-containing preparations from immunized cows are regarded as pharmaceuticals in the USA and EU, while in many countries their regulatory status is not yet defined. On the contrary, normal colostrum or immunoglobulin-containing preparations made thereof are in many countries regarded as food or dietary supplements (Hoerr & Bostwick, 2002; Mehra *et al*., 2006; Young *et al*., 2007).

Since late 1980s, a great number of clinical studies have demonstrated that immune milk preparations can be effective in prevention of diseases caused by different pathogenic microbes, such as rotavirus, EPEC, *C. albicans*, *Clostridium difficile*, *Shigella flexneri*, *Streptococcus mutans*, *Cryptosporidium parvum* and *H. pylori*. Since these studies have been reviewed earlier (Korhonen *et al*., 2000; Hammarström & Weiner, 2008), the protocols and results are not discussed in detail. Briefly, in most studies orally administered cow colostral or milk immunoglobulins have proved to be effective in prevention of orally mediated infections. In treatment of already established infections, promising therapeutic effects have been reported in such diseases in which the infection is maintained through reattachment and reinfection inside the gastrointestinal tract or oral cavity, and in which secretion of toxins or other inflammatory compounds are involved, that can be neutralized by the specific immunoglobulins. Such infections are caused by, for example, *S. mutans*, enterotoxigenic *E. coli*, *C. albicans*, *S. flexneri*, rotaviruses and *C. parvum*.

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The dissemination of emerging antibiotic-resistant strains of pathogenic bacteria is causing a rapidly increasing global problem. Development of suitably designed immune milk products may offer solutions for protecting people against orally transmitted pathogens carrying antibiotic resistance factors. A few immune milk products are on the market in some countries but the unclear regulatory status of these products has emerged as a constraint for global commercialization.

8.4 BIOACTIVE PEPTIDES

Proteins are made up of 20 different amino acids arranged into diverse conformations, giving each protein molecule its own unique structure and function. Amino acids can be cleaved at specific sites on the proteins to yield peptide sequences of different sizes. Bioactive peptides are considered protein fragments that, upon hydrolysis by proteolytic enzymes or fermentation, impart positive functions or conditions that influence human health (Kitts & Weiler, 2003). The activity of peptides is based on their inherent amino acid composition and sequence. The size of active sequences may vary from two to twenty amino acid residues, and many peptides are known to display multifunctional properties. Milk proteins are considered the most important source of bioactive peptides, and at present more than 200 biologically active peptide sequences have been identified from caseins and whey proteins of most dairy animals. The best characterized include antihypertensive, antithrombotic, antimicrobial, antioxidative, immunomodulatory, and opioid peptides. Their production, functionality and potential applications have been reviewed in many articles and books (Korhonen & Pihlanto, 2006; Phelan *et al*., 2009; Nagpal *et al*., 2011).

8.4.1 Production systems

Bioactive peptides are encrypted as inactive within the amino acid sequence of their parent protein molecule and can be released in the following ways: (i) hydrolysis by digestive enzymes, (ii) fermentation of milk with proteolytic starter cultures, and (iii) proteolysis by microbial or plant-derived enzymes (Korhonen & Pihlanto, 2007; Vercruysse *et al*., 2009). In many studies these methods have been combined successfully to generate novel functional peptides. The gastrointestinal enzymes pepsin, trypsin and chymotrypsin have been shown to release a great number of antihypertensive peptides, casein phosphopeptides (CPPs), antibacterial, antioxidative, immunomodulatory, and opioid peptides both from caseins and the whey proteins α-LA, β-LG and GMP (López-Fandino *et al*., 2006; López-Expósito *et al*., 2007; del Mar Contreras *et al*., 2009). Also, commercial proteolytic enzymes, such as alcalase, Flavourzyme, thermolysin and subtilisin and other proteases, have been employed to release various bioactive peptides from both caseins and whey proteins (Pihlanto-Leppälä *et al*., 2000; Otte *et al*., 2007; Ortiz-Chao *et al*., 2009).

Many lactic acid bacteria (LAB) and probiotic strains are highly proteolytic and the release of different bioactive peptides from milk proteins by means of microbial fermentation is now well documented (FitzGerald & Murray, 2006; Hayes *et al*., 2007a, b). The bioactivity of released peptides depends on the strain used and fermentation time and conditions. A great number of dairy cultures and probiotic strains have been applied to study the release of such peptides but *Lactobacillus helveticus* strains in particular have proven highly prominent in releasing antihypertensive peptides. The best-studied peptides are the ACE-inhibitory tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) (López-Fandino *et al*., 2006; Murray & FitzGerald, 2007; Ricci *et al*., 2010). These amino acid sequences are present in β- and κ-casein fractions and GMP and can be released by both microbial and pepsin treatments. Yogurt bacteria, cheese starter bacteria and commercial probiotic bacteria have also been shown to produce antihypertensive, antioxidative, antimicrobial, and immunomodulatory peptides in milk during fermentation (Gobbetti *et al*., 2004; Pihlanto *et al*., 2010; Gonzalez-Gonzalez *et al*., 2011). Virtanen *et al*. (2006) studied the production of antioxidant activity during fermentation of milk with 25 LAB strains. It was demonstrated that the development of antioxidant activity was strain-specific, with *Leuconostoc mesenteroides* subsp. *cemoris* strains, *Lactobacillus jensenii* (ATCC 25258), and *Lactobacillus acidophilus* (ATCC 4356) showing the highest activity. The activity correlated positively with the degree of proteolysis, suggesting that peptides were responsible for the antioxidative property. Donkor *et al*. (2007) studied the proteolytic activity of several dairy cultures and probiotic strains (*Lb. acidophilus*, *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus casei*) as determinants of growth and *in vitro* ACE-inhibitory activity in milk fermented with these single cultures. All the cultures released ACE-inhibitory peptides during growth with a *B. longum* strain, the probiotic *Lb. acidophilus* strain showing the strongest ACE-inhibitory activity. Pihlanto-Leppälä *et al*. (1998) demonstrated that fermentation of milk with starter cultures alone was not enough to generate ACE-inhibitory activity, but a further digestion with pepsin and trypsin was necessary. It can be expected that similar events may happen also under *in vivo* conditions in the gastrointestinal tract when ingesting fermented dairy products. In a recent study, Paul and Somkuti (2009) demonstrated that antimicrobial and hypotensive polypeptides released from LF by pepsin remain mostly intact at pH 4.5 when added into yogurt at the end of the fermentation process. Thus, the

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health-promoting qualities of fermented liquid or semi-solid dairy foods may be increased by supplementation with bioactive peptides.

For enrichment and isolation of bioactive peptides from the hydrolyzates of milk proteins, membrane filtration systems, chromatographic methods and selective precipitation have been developed in recent years and many companies now manufacture specific peptide concentrates.

8.4.2 Functionality

A great number of *in vitro* and experimental studies conducted since the 1980s have demonstrated the wide functionality of bioactive peptides released from cow milk proteins. These studies suggest that such peptides when ingested are able to deliver physiological effects in the body and can affect, for example, gastrointestinal, cardiovascular, endocrine, immune, and nervous systems. Until now, a majority of animal model and human studies were concerned with antihypertensive, mineral-binding, and anticariogenic peptides. Growing research interest is nowadays focused on dietary peptides which could regulate satiety, postprandial insulin response, and mood. In the following sections, recent studies about the functionality of a few specific groups of milk protein-derived bioactive peptides are described.

8.4.2.1 Antihypertensive

Peptides possessing the capacity to lower blood pressure are the most studied ones among the milk protein-derived peptides. In total more than 150 antihypertensive peptides have been identified in different milk proteins from cow, buffalo, goat, and sheep. The antihypertensive capacity of many milk peptides has been indicated in *in vitro* and rat model studies (Murray & FitzGerald, 2007; Jäkälä & Vapaatalo, 2010; Ricci *et al*., 2010). In these studies, the tripeptides VPP and IPP have proven the most effective (Nakamura *et al*., 1995; Jauhiainen *et al*., 2005; de Leeuw *et al*., 2009). In human studies moderate or significant reduction of blood pressure has been observed in mildly hypertensive subjects after consumption of fermented dairy products or tablets containing these peptides (Seppo *et al*., 2003; Hirota *et al*., 2007; Boelsma & Kloek, 2009; Usinger *et al*., 2010). In the above studies, reductions of 1.5–14.0 mmHg for systolic blood pressure (SBP) and 0.5–6.8 mmHg for diastolic blood pressure (DBP) compared with placebo have been recorded. Effective dosages of lactotripeptides (VPP and IPP) range from 3.07 to 52.5 mg/day. Blood pressure-lowering effects of lactotripeptides have typically been observed after 4–6 weeks of treatment. Maximum blood pressure reductions of approximately 13 mmHg SBP and 8 mmHg DBP have been reached after active treatment for 8–12 weeks (Phelan & Kerins, 2011). Absorption from the gastrointestinal tract into circulation and a dose-dependent antihypertensive effect *in vivo* of VPP and IPP have been established in rat model and human studies (Hata *et al*., 1996; Aihara *et al*., 2005; Jauhiainen *et al*., 2007). Foltz *et al*. (2007) showed in a placebo-controlled, full crossover intervention study that IPP was absorbed intact from a fermented milk drink into the circulation of healthy human subjects. On the other hand, several recent studies have not established any significant effect of drinks containing ACE-inhibitory peptides (Lee *et al*., 2007) or of VPP and IPP peptides on blood pressure in human subjects with mild hypertension (van der Zander *et al*., 2008; Engberink *et al*., 2009; van Mierlo *et al*., 2009). Also, the three recent meta-analyses have resulted in inconclusive results about the efficacy of milk-derived peptides in reduction of hypertension (Pripp, 2008; Xu *et al*., 2008; Usinger *et al*., 2009). A recent study (Turpeinen *et al*., 2009) has suggested that the beneficial effects of tripeptides on cardiovascular functions can be combined with other dietary components, for example with cholesterol-lowering plant sterols. The effects of a spread containing IPP, VPP and plant sterols were studied in subjects with mild hypertension and elevated low-density lipoprotein (LDL) cholesterol; 62 subjects consumed 20 g/day of the spread containing 4.2 mg milk peptides and 2 g plant sterol esters or placebo for 10 weeks. A significant decrease was seen in SBP ($P=0.026$), but not in DBP ($P=0.53$). Also, total cholesterol and LDL cholesterol decreased significantly, whereas high-density lipoprotein (HDL) cholesterol and triacylglycerols remained unchanged. The results suggest that a spread containing bioactive milk peptides and plant sterols has a beneficial effect on two major cardiovascular risk factors, blood pressure and plasma lipids, in hypertensive dyslipidemic subjects.

Apart from ACE-inhibitory activity, the lactotripeptides VPP and IPP have been shown to exert a beneficial effect on arterial stiffness of hypertensive rats (Jäkälä *et al*., 2009) and mildly hypertensive human subjects (Jauhiainen *et al*., 2007). Another possible mechanism for the antihypertensive action of milk protein-derived peptides is stimulation of nitric oxide production by endothelial cells. A single whey-derived peptide that increased endothelial nitric oxide synthesis *in vitro*, improved *in vivo,* after a 2-week supplementation, both conduit and resistance vascular responses in healthy individuals with normal endothelial function. However, whether better maintenance of NO· after the peptide ingestion contributed to the enhanced vascular responses in this study remains unclear (Ballard *et al*., 2009). In view of the great public health importance of hypertension, the potential beneficial impact of the antihypertensive effects of milk-derived peptides deserves further research.

8.4.2.2 Antimicrobial

A variety of antimicrobial peptides (AMPs) are formed from bovine milk proteins when digested by proteases in the gastrointestinal tract. Together with phagocytosis, the production of AMPs is considered to be the most ancient

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mechanism of immunity. Adsorption of AMPs onto the bacterial cell membrane by electrostatic attraction and their subsequent aggregation and integration into the lipid bilayer results in local membrane thinning. AMPs finally insert into the membrane, leading to the formation of ion channels, transmembrane pores, or extensive membrane rupture (Wiesner & Vilcinskas, 20110. In milk protein-derived peptides the antimicrobial activity seems to be mainly correlated with the net positive charge of the peptides (Meisel, 2004).

Caseicidins are polyanionic low molecular mass AMPs derived from both α_{s1}-casein and κ-caseins as a result of proteolysis by chymosin at pH 6–7. Caseicidins exhibit antimicrobial activity against staphylococci, *Bacillus subtilis* and *Streptococcus pyogenes* (Meisel, 1997). Isracidin is released from the N-terminus of α_{s1}-casein by chymosin. Isracidin is effective against a variety of Gram-negative and Gram-positive bacteria and has been shown *in vivo* to be effective against a lethal *S. aureus* and *C. albicans* infection in a mouse model (Chan & Li-Chan, 2006). Acid treatment of α_{s2}-casein releases a 39-amino acid sequence f(150–188), casocidin-I, which inhibits the growth of *E. coli* and *Staphylococcus carnosus*. Pepsin digestion of α_{s2}-casein releases two other cationic domains f(164–179 and 183–207) with more potent antimicrobial activity than casocidin-I. GMP inhibits the growth of the oral pathogens *Streptococcus mutans* and *Porphyromonas gingivalis* (Malkoski *et al*., 2001).

Digestion of cow milk α-LA with trypsin produces two AMPs, and with chymotrypsin one AMP having the most activity against Gram-positive bacteria, especially *B. subtilis* (Chan & Li-Chan, 2006). Hydrolysis of β-LG with trypsin results in four AMPs that inhibit the growth of only Gram-positive bacteria, *B. subtilis* being the most sensitive. Unlike most other AMPs these four are all negatively charged (Pellegrini *et al*., 2001).

LF displays some characteristics of classical AMPs, such as its rather non-specific mode of action. Gastric pepsin cleaves from the N-terminal region of LF a 25-amino acid AMP, lactoferricin f(17–41). There is direct evidence for the generation of lactoferricin in human stomach after ingestion of cow milk LF. Lactoferricin has variable antimicrobial activity against a wide range of both Gram-negative and Gram-positive bacteria, fungi, molds, and protozoa (Weinberg, 2007; Jenssen & Hancock, 2009). Lactoferricin is particularly bactericidal against EPEC and has been found to be active against clinical isolates of enterohemorrhagic *E. coli* O157:H7. Cow milk lactoferricin is in *in vitro* conditions able to induce apoptosis in *L. monocytogenes*-infected THP-1 cells and in Caco-2 epithelial cells. Furthermore, LF can be processed into synthetic peptides such as lactoferricin B f(17–30) and lactoferrampin f(265–284) which have been found to be even more active than lactoferricin and act synergistically with antibiotics against multidrug-resistant *S. aureus* and EPEC (Flores-Villaseñor *et al*., 2010). In the course of evolution, killing of pathogens by producing AMPs has proven to be an effective defense strategy, which should not be ignored at the present time of rapid global spread of multidrug-resistant pathogens.

8.4.2.3 Immunomodulatory

Intact whey proteins α-LA and β-LG have recently been reported to prime human neutrophils to inflammatory cells, seen as increased chemotaxis, degranulation, and oxygen radical production due to secondary stimuli (Rusu *et al*., 2009, 2010). GMP enhanced the priming effect of α-LA and β-LG. They also stimulate synthesis of proinflammatory cytokines in epithelial-like Caco-2 cells (Ustunol & Wong, 2010), and lymphocyte proliferation (Gauthier *et al*., 2006b). In a neonatal calf such an immune cell boosting may be temporarily beneficial but otherwise it is an undesired effect. According to current knowledge on immunity and widespread diseases, a health-promoting food should not prime or stimulate leukocytes.

Inflammatory leukocytes play a central role in the pathogenesis of atherosclerosis and, along with obesity, in the development of metabolic syndrome and type 2 diabetes. Food compounds having anti-inflammatory properties are likely to have beneficial effects with regard to cardiovascular disease and type 2 diabetes. However, increased IL-6 production may have also beneficial health effects, since IL-6 possesses several anti-inflammatory activities, such as downregulation of LPS-induced TNF-α mRNA expression. Enhanced mucosal SIgA response is considered to reduce allergic and inflammatory responses and inhibit leaking of antigens in mucosa to GALT.

The casein-derived peptides BCM-7 and β-casokinin-10 have been shown to have, depending on the concentration, either suppressive or stimulatory effects on proliferation of human lymphocytes (Meisel, 2004; Gauthier *et al*., 2006b). GMP was reported to have anti-inflammatory activity on rats in an experimental dextran sulfate sodium-induced colitis model. GMP pretreatment (500 mg/kg daily) starting 2 days before the challenge normalized colonic cytokine expression (López-Posadas *et al*., 2010). The immunological activities of LF and lactoferricin are reviewed separately (see Lactoferrin).

Probiotic bacteria may produce bioactive peptides from milk proteins during the fermentation process. Such peptides may partially contribute to the beneficial effects attributed to probiotics. Formulas containing whey-derived peptides and fermented milk have been reported to reduce experimental inflammation of the gut

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(Nakamura *et al*., 2011) and liver (Kume *et al*., 2012) in mice. In these studies systemic proinflammatory cytokine levels were significantly lower in mice groups fed whey-derived peptides and fermented milk. Milks fermented with *Lb. helveticus* have been shown in mouse models to improve SIgA secretion in intestinal mucosa *in vivo*, and to enhance the immune response against subcutaneous fibrosarcomas (LeBlanc *et al*., 2002) and synthesis of IL-6 and anti-inflammatory IL-2 and IL-10 (Vinderola *et al*., 2007). Peptide fractions from *Lb. helveticus*-fermented milk inhibited IL-6 and TNF-α production and respiratory burst activity of LPS-primed human THP-1 promonocytes (Tompa *et al*., 2011).

8.4.2.4 Mineral binding

Calcium-binding phosphopeptides have been studied for potential health effects such as enhancement of calcium bioavailability, but the results obtained in animal and human studies have been inconclusive (Meisel, 1997; Meisel & Fitzgerald, 2003). Instead, the anticariogenic effect of some CPPs is well documented in animal and human studies (Morgan *et al*., 2008) and such specific peptides have been commercialized. CPPs may also stimulate the local immune system. Mice fed a CPP preparation had a higher level of serum and intestinal antigen-specific IgA than those fed the control diet (Otani *et al*., 2000).

8.4.3 Occurrence in dairy products

It is now well documented that bioactive peptides are formed in the manufacturing and maturation processes of various fermented dairy products as a result of proteolytic action by added microbial cultures. In screening studies a great variety of bioactive peptides has been found in fermented dairy products, such as yogurt, sour milk, dahi, kefir, quark, and different types of cheese, for example Cheddar, Edam, Emmental, Gouda and many Italian varieties (FitzGerald & Murray, 2006; Gobbetti *et al*., 2007). The occurrence, specific activity, and amount of bioactive peptides in fermented dairy products depend on many factors, such as type of starters used, type of product, time of fermentation, and storage conditions (Ardö *et al*., 2007; Ong *et al*., 2007; Bütikofer *et al*., 2008). Ong and Shah (2008) demonstrated that Cheddar cheeses made with the addition of probiotic *Lb. casei* 279, *Lb. casei* LAFTI®L26 or *Lb. acidophilus* LAFTI®L10 had significantly higher ACE-inhibitory activity than those without any probiotic adjunct. These probiotic adjuncts are known to improve proteolysis and enhance flavor during Cheddar cheese ripening. It is noteworthy that in fermented dairy products peptides with different bioactivities (e.g., calcium-binding, antihypertensive, antioxidative, immunomodulatory, antimicrobial) can be found at the same time. The formation of peptides can be regulated to some extent by starter and adjunct cultures used, but the stability of desired peptides during storage seems difficult to control. For example, ACE-inhibitory activity increases during cheese maturation, but decreases when the proteolysis exceeds a certain level (Bütikofer *et al*., 2007, 2008). Apart from generation during the ripening process, more bioactive peptides are likely to be formed in the gastrointestinal tract upon ingestion of a fermented dairy product. Ardö *et al*. (2009) evaluated the impact of heat-treated *Lb. helveticus* CNRZ 303 culture on the formation, accumulation and hydrolysis of bioactive peptides during ripening of semi-hard cheese. During ripening, the formation of antioxidative peptides and phosphopeptides were observed and they were further hydrolyzed by *Lb. helveticus* enzymes. Also, ACE-I-inhibitory peptides accumulated during the ripening period of 9 months but no hydrolysis by *Lb. helveticus* enzymes was noted. Further increased ACE inhibition was observed when the cheese samples were digested *in vitro* by Corolase PP (Röhm, Germany) enzyme preparation simulating gastrointestinal enzymes. Hernández-Ledesma *et al*. (2004) evaluated the ACE-inhibitory activity of commercial fermented milks and fresh cheeses and found that most of these products showed moderate ACE-inhibitory activity. The ACE-inhibitory activity of these commercial products remained stable or increased after simulated gastrointestinal digestion with pepsin and Corolase PP. Several ACE-inhibitory peptides have been identified in other fermented milks, such as kefir made from caprine milk (Quiros *et al*., 2005), koumiss from mare's milk (Chen *et al*., 2010), and yogurt from ovine milk (Chobert *et al*., 2005). The potential *in vivo* effect of fermented cheese or fermented milk products containing naturally formed antihypertensive peptides on blood pressure of hypertensive subjects remains to be studied.

Calcium-binding CPPs have been identified in casein hydrolyzates, milk-based infant formula, and fermented dairy products such as cheese and yogurt (Miquel *et al*., 2005; Dupas *et al*., 2009).

8.4.4 Applications

Commercially marketed products which contain milk protein-derived bioactive peptides are now available in many countries. These products include dairy and fruit-based drinks, confectionery, chewing gum, pastilles, and capsules. They are claimed to possess antihypertensive, anticariogenic, mineral-binding, or stress-relieving properties (Hartmann & Meisel, 2007; Korhonen, 2009b,c; Phelan *et al*., 2009). Examples of these commercial ingredients and their applications are listed in Table 8.2. So far, the best-studied peptide products are the fermented milk

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Table 8.2. Examples of commercial bioactive milk protein hydrolyzates and peptides.

Brand name	Type of product	Functional component	Suggested or proven function	Manufacturer
Calpis	Sour milk	Val-Pro-Pro, Ile-Pro-Pro, derived from β-casein and κ-casein	Reduction of blood pressure	Calpis Co., Japan
Evolus	Fermented milk	Val-Pro-Pro, Ile-Pro-Pro, derived from β-casein and κ-casein	Reduction of blood pressure	Valio Ltd., Finland
Recaldent	Chewing gum	Calcium casein peptone-calcium phosphate	Anticariogenic	Cadbury Adams, USA
ProDiet F200/ Lactium	Flavored milk drink, confectionery, capsules	α_{s1}-casein f(91–100) (Tyr-Leu-Gly-Tyr-Leu-Glu-Gln-Leu-Leu-Arg)	Relief of stress symptoms	Ingredia, France
BioZate	Hydrolyzed whey protein isolate	β-lactoglobulin fragments	Reduction of blood pressure	Davisco, USA
BioPURE-GMP	Whey protein isolate	κ-casein f(106–169) (glycomacropeptide)	Satiety regulation through CCK*, anticariogenic	Davisco, USA
Vivinal Alpha	Ingredient	α-lactalbumin-rich whey protein hydrolyzate	Aids relaxation and sleep	Borculo Domo Ingredients (BDI), Netherlands
Praventin	Food supplement/ capsule	Lactoferrin-enriched whey protein hydrolyzate	Helps reduce acne	DMV International, Netherlands
Dermylex	Food supplement/ tablet	Whey protein extract XP-828L	Helps reduce symptoms of mild to moderate psoriasis	Advitech Inc., Canada
Insulvital	Ingredient	Casein hydrolyzate	Helps regulate blood sugar peaks after a meal	Wild Co., Germany
Immunel	Ingredient	Milk peptides	Reduces inflammation and promotes healing in the digestive tract	Wild Co., Germany
Tegricel	Ingredient	Milk peptides	Supports and balances total immunity	Wild Co., Germany

* CCK, cholecystokinin.

Source: based on data from Korhonen (2009b).

products Calpis® and Evolus®, which are targeted to subjects having mild hypertension. The blood pressure-reducing effects of both products have been established in many human studies (Jäkälä & Vapaatalo, 2010; Phelan & Kerins, 2011). These products contain the two ACE-inhibitory tripeptides VPP and IPP. The Japanese product Calpis® is fermented with a culture containing *Lb. helveticus* and *Saccharomyces cerevisiae* and the Finnish product Evolus® is produced using a *Lb. helveticus* LBK-16H strain in milk fermentation.

Many CPP preparations based on casein hydrolyzates have been commercialized for various purposes, such as dental care (Recaldent®). Other interesting applications of bioactive peptides include Lactium®, Vivinal Alpha® and Cysteine peptide®, which claim to deliver stress-relieving effects. PeptoPro® is targeted to improve athletic performance and recovery from physical exercise. Insulvital™ is a casein hydrolyzate and is claimed to help regulate blood sugar peaks after a meal when taken orally in connection with a meal. Tegricel™ is a product made from milk

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peptides and other milk bioactives and is claimed to reduce inflammation and promote healing in the digestive tract. Immunel™ is a blend of milk peptides that are suggested to support and balance total immunity. Apart from cow milk, the bioactive proteins or peptides from other domesticated dairy animals are currently exploited industrially to a very limited extent. This can be ascribed to the fact that relatively little research has been done on these components and that there are no suitable technologies for isolation and purification of such components from colostrum or milk of other species other than the cow. This situation is expected to change in the future as the demand grows for bioactive ingredients derived from other species. In particular, such special applications can be envisaged in the area of clinical and medical nutrition, biomedication and sports nutrition.

8.5 OTHER MINOR PROTEINS

A variety of minor proteins/peptide compounds have been found in milk and especially in colostrum. Cow milk contains glycosylation-dependent cell adhesion molecule (GlyCAM)-1, also known as lactophorin and PP3. It consists of a diverse group of glycoproteins or glycopeptides and is a mucin-like antibacterial component expressed by udder epithelial cells. The soluble form of this protein is found in milk and it is speculated that it is involved in lubrication and protection of the intestinal tract and may also have an antibacterial function (Senda *et al*., 2011).

The milk fat globule membrane (MFGM) contains unique proteins. Many of these proteins, such as mucin, MUC1, lactadherin, sphingomyelin, butyrophilin, xanthine oxidase and alkaline phosphatase, have been shown to exert antimicrobial activities. Thus, MFGM protein concentrates may protect against viral and bacterial diarrhea (Spitsberg, 2005). In a recent double-blind study, an MFGM protein-enriched complementary food was given to Peruvian infants twice daily at a dose of 40 g/day for 6 months. Consumption of MFGM protein food significantly reduced episodes of bloody diarrhea as compared with the control food made of skim milk protein (Zavaleta *et al*., 2011).

Unprocessed colostrum and MFGM contain CD14 molecules (Hettinga *et al*., 2011). These are part of the Toll-like receptor 4 complex that detects bacterial LPS and subsequently activates the innate immune system. Soluble milk CD14 belongs to "pattern recognizing receptors" that facilitate the intestinal responses to specific microbial motifs. CD14 is therefore considered to present, together with SIgA, an important defense mechanism for protection against pathogen invasion (Labeta *et al*., 2002).

Hettinga *et al*. (2011) analyzed pooled milk samples from 30 clinically healthy cows using a shotgun proteomics approach focusing on defense proteome. A total of 269 proteins were identified from cow milk and 51 of these were related to the host defense system; 192 proteins were identified from "milk serum" and 232 proteins in MFGM. Colostrum seems to contain more different low abundance proteins than milk (Senda *et al*., 2011), which is probably important for immune defense, health and development of calves during their first days of life. Milks of other species than cow provide an abundant source of yet undiscovered molecules. There remains an immense amount of scientific research to be done in finding out the potential of these compounds. However, many of them may in the future provide substantial added value as ingredients of health-promoting products.

8.6 CONCLUSIONS

The current global interest in developing functional foods provides a timely opportunity to exploit the broad arsenal of native bioactive milk components for promotion of human nutrition and health. This approach has been facilitated by the rapidly increasing knowledge about the biological properties and mechanism of action of major milk proteins, bioactive peptides, and growth factors. It is obvious that more minor molecules with specific bioactivities will be exploited in the coming years and also new bioactive molecules will be discovered with the help of modern analytical and biomolecular methods. Some examples of such biomolecules are described briefly in this chapter. Commercial applications based on these components can be developed now more easily using advanced industrial or semi-industrial scale fractionation and isolation techniques. Modern non-thermal, clean and green technology based methods are well suited for manufacturing heat-sensitive ingredients from these sources. Biomining of specific milk molecules and marketing them as ingredients is now emerging as a new lucrative business for the dairy industries and specialized bio-industries. Examples of product development are specific casein and whey protein hydrolysates and products enriched with LF or bioactive peptides. Apart from cow, colostrum and milk from other animals, e.g. buffalo, goat, sheep, mare and camel, may prove as a good source for specific ingredients with targeted use for health promotion or reduction of certain disease risks. For example, camel milk has been claimed as beneficial for diabetic people though the active compounds in camel milk have not been identified (Agrawal *et al*., 2011). Mare's milk has been used traditionally in some countries for substituting human milk or treating patients suffering from e.g. tuberculosis, peptic ulcer or chronic hepatitis (Sheng & Fang 2009). Again, according to many clinical studies, goat milk is suitable for children allergic to cow milk and people suffering from various allergic symptoms (Park 2009). The increasing knowledge about

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bioactive components in milks amongst different species and breeds allows the development of products for consumers with special needs. Such products could be targeted to people suffering from e.g. allergy, milk intolerance, type 1 diabetes or AIDS. Also, they could be designed to prevent diet-related chronic diseases, e.g. obesity and type two diabetes and degeneration of cognitive functions. An emerging research field is nutrigenomics which could focus on studying the effects of milk proteins and peptides on the functions of animal and human genomes and susceptibility to diseases. Research in this field may in the future open new opportunities for optimal exploitation of milk proteins from different species for human nutrition and wellbeing.

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9
Milk Minerals, Trace Elements, and Macroelements

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9.1 INTRODUCTION

Proteins, lipids, and lactose are the main macronutrients in milk and dairy products that contribute significantly to nutritive and biological values for human nutrition and health. Milk and dairy products also contain essential minerals at different concentrations. They are interesting for biochemical, technological, and nutritional reasons because they contribute significantly to different vital functions of the human body. They are classified as macroelements (Ca, P, Mg, Na, K, Cl) and trace elements (Fe, Cu, Zn, Se, Mn, I, F, Cr, Pb, Cd, Co, Mo, As, Ni, Si, B). Their quantities, chemical forms, and associations in different dairy products have been well analyzed and are very interesting for human health. Some of them can also be considered toxic.

The objective of this chapter is to describe the knowledge on a selected number of 10 minerals (Ca, P, Mg, Na, K, Cl, Fe, Cu, Zn, and Se) and mainly in cow milk. Their importance and biological roles for the human organism are briefly described and then their concentrations, chemical forms, and locations in different dairy products are discussed, as has been reviewed by several authors (Flynn, 1992; Flynn & Cashman, 1997; Gaucheron 2004; Cashman, 2011a, b). Other minerals (Mn, I, F, Cr, Pb, Cd, Co, Mo, As, Ni, Si, B) and minerals in the milk of other species are also discussed briefly as far as the scant literature allows.

9.2 MACROELEMENTS IN MILK AND DAIRY PRODUCTS FROM THE COW

9.2.1 Calcium (Ca)

9.2.1.1 Calcium in the human organism and biological roles

In the human organism, Ca is the most abundant mineral (Table 9.1). It exists in different locations and under different forms. It can be solid, dissolved, free, or associated with other compounds. Ca plays an important structural role for bones and teeth. In these cases, Ca is associated with phosphate to form a crystallized salt named hydroxyapatite. It provides rigidity, strength, and hardness. Furthermore Ca contributes to blood clotting, neurotransmission, neuromuscular excitability, cardiac conduction, stimulation of the sympathetic system, intestinal motility, cell signaling (by acting as a second messenger), fertility, cell proliferation, muscle contraction, secretion or cell death, hormone secretion, and structure and activation of Ca-proteins.

9.2.1.2 Contents and chemical forms of Ca in milk and dairy products

Ca is one of the major minerals in milk and dairy products (Tunick, 1987; Gaucheron, 2004). In cow milk, its concentration is about 1200 mg/L. This concentration is considered relatively constant but slight variations can be observed

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Table 9.1. Mineral composition of 70-kg human and biological roles.

Element	Per cent	Mass (kg)	Biological roles
Ca	1.4	1.0	Bone and teeth constitution, blood pressure, muscle contraction, blood clotting, cofactor of enzymatic systems, obesity
P	1.1	0.78	Bone and teeth constitution, blood pH, component of biological molecules (RNA, DNA, phospholipids, ATP), metabolism
K	0.25	0.14	Blood pressure, muscle contraction, ionic equilibrium
Na	0.15	0.100	Blood pressure, muscle contraction, ionic equilibrium
Cl	0.15	0.095	Blood pressure, muscle contraction, ionic equilibrium
Mg	0.05	0.019	Cofactor of enzymatic systems, phosphorylation, DNA transcription, protein synthesis, neuromuscular transmission, muscle contraction
Fe	0.006	0.0042	Component of heme in hemoglobin, myoglobin, cytochromes; cofactor of enzymatic systems
F	0.0037	0.0026	Component of teeth
Zn	0.0032	0.0023	Cofactor of enzymatic systems playing roles in DNA, RNA, and protein synthesis; component of insulin
Si	0.002	0.0010	Structure of bone
Pb	0.00017	0.00012	Toxic
Cu	0.0001	0.000072	Cofactor of enzymatic systems, role in respiratory chain (cytochrome C oxidase), catabolism of dopamine
B	0.000069	0.000018	Not clearly identified
As	0.000026	0.000007	Toxic
Se	0.000019	0.000015	Cofactor of enzymatic systems (glutathione peroxidase), antioxidant
Mn	0.000017	0.000012	Cofactor of enzymatic systems
I	0.000016	0.000020	Component of thyroid hormones (T_3, T_4)
Ni	0.000014	0.000015	Component of urease
Mo	0.000013	0.000005	Cofactor of enzymatic systems (molybdenum oxotransferases, xanthine oxidase, and sulfite oxidase)
Cr	0.0000024	0.000014	Not clearly identified
Co	0.0000021	0.000003	Component of vitamin B_{12}

Source: based on data from http://en.wikipedia.org/wiki/Composition_of_the_human_body.

in some cases. Thus, the Ca content is higher in milks rich in proteins. For example, the milk from Normande cows has a higher Ca content than milk from Frisonne, Pie rouge, and Holstein cows. The concentration of Ca also varies with stage of lactation, and the most important changes in composition occur at around parturition; thus the Ca concentration in colostrum is much higher than that of normal milk and that near the end of lactation. Concerning its location, 99% of the Ca is in the skim milk where it is distributed between micellar (about 800 mg/L) and aqueous phases (about 400 mg/L). In the aqueous phase, Ca is free (ionic Ca^{2+}) and associated with citrate and inorganic phosphate to form salts. The aqueous phase is considered as saturated in Ca phosphate. In this aqueous phase, a small part of Ca is also associated with whey proteins like α-lactalbumin and osteopontin. In the micellar phase, Ca is in interaction with organic phosphate (phosphoseryl residues of casein molecules) and inorganic phosphate.

These micellar associations between Ca and phosphates are formed as small granules (named nanoclusters) having a diameter close to 3 nm (McGann *et al*., 1983; Holt, 1993, 1997), but their exact chemical composition is not well known. There is a thermodynamic mineral equilibrium between the aqueous and micellar phases which is relatively well described (Gaucheron, 2004; Mekmene *et al*., 2009, 2010). Ca and inorganic phosphate present in one phase can be transferred to the other one depending on physicochemical conditions (De la Fuente, 1998; Gaucheron *et al*., 2004). One of the most important modifications is the biological acidification during fermentation of lactose to lactic acid. During this step, Ca is solubilized and transferred to the aqueous phase of acidified milk (Le Graët & Brulé, 1993). Depending on the type of cheeses, the curd is more or less acidified and the aqueous phase more or less removed by drainage. Consequently, the Ca content in cheeses depends on the cheese technology used. As shown in Table 9.2,

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Table 9.2. Concentration* of minerals in different dairy products.

	Ca	Mg	Na	K	P	Zn	Mn	Fe	Cu	Se	I
Milk powders											
Whole milk powder	912	85	371	1330	776	3.34	0.04	0.47	0.08	16.3	
Whole milk powder	947	88	322	1195	706	3.33	0.043	0.45	0.15	7.8	71
Skim milk powder	1257	110	535	1794	968	4.08	0.02	0.32	0.041	27.3	
Skim milk powder	1254	115	420	1468	990	3.96	0.057	0.433	0.2	10.5	85
Semi-skimmed milk powder	1034	97	347	1330	829	3.7	0.048	0.345	0.2	8.8	80
Liquid milks											
Skim milk	125	11	42	156	101	0.42	0.003	0.03	0.013	3.1	
Partially skimmed milk 1% fat	119	11	44	150	95	0.42	0.003	0.03	0.01	3.3	
Goat whole milk	134	14	50	204	111	0.3	0.018	0.05	0.046	1.4	
Goat raw whole milk	107	12.9	36.6		77.6	0.334		0.2	0.1	4	25
Sheep whole milk	188	18	38	153	135	0.6	0.013	0.09	0.043	2.4	29.9
Sheep whole milk	193	18	44	137	158	0.54	0.018	0.1	0.046	1.7	
Pasteurized skim milk	132	134	50	170	111	0.35	0.005	0.09	0.01	1	14.4
Semi-skimmed pasteurized milk	118	10.6	43.5	166	83.1	0.398	0.002	0.05	0.006	1.1	10.6
Whole pasteurized milk	118	10.6	44.1	157	84.2	0.37	0.002	0.05	0.008	2	9.4
UHT whole milk	117	10	43.9	150	84.2	0.38	0.002	0.05	0.007	2.2	9.2
UHT skim milk	113	10.6	41.8	173	88.8	0.41	0.0016	0.05	0.003	0.8	12.7
UHT semi-skimmed milk	115	11.6	49.6	165	85.7	0.51	0.001	0.16	0.003	0.9	10.6
UHT whole goat milk	120	13	43	188	99	0.38	0.01	0.08	0.04	1.7	19
UHT fluid cream 30% fat	70	7	35	104	65	0.5		0.125	0.01	1.3	11
Concentrated whole milk	264	25	114	287	213	0.89	0.016	0.15	0.05	1.9	28
Concentrated sweet milk	284	26	127	371	253	0.94	0.006	0.19	0.015	14.8	
Whole homogenized pasteurized milk 3.3% fat	113	10	40	144	91	0.4	0.003	0.003	0.011	3.7	
Cream/butter/buttermilk											
Salted butter	24	2	576	24	24	0.09		0.02		1	
Unsalted butter	24	2	11	24	24	0.09	0.004	0.02	0.016	1	
Light butter	16	3.6	260	31	31	0.01	0.01	0.05	0.005	1	1.5
Fresh cream 15/20%	103	9.5	41	124	74	0.34	0.007	0.22	0.01	1.3	10.6
Buttermilk	1184	110	517	1592	933	4.02	0.023	0.3	0.111	20.3	
Yogurts											
Yogurt with skim milk	127	11	48	172	94	0.44	0.0083	0.1	0.008	2.2	15
Yogurt with skim milk	143	12.8	53.2	178	99	0.63	0.003	0.13	0.02	1.5	20
Yogurt with semi-skimmed milk	114	10	38	150	82	0.362	0.002	0.13	0.004	1.6	11
Yogurt with skim goat milk	112	13	36	159	103	0.4		0.2	0.01		

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Yogurt with semi-skimmed milk and fruits	111	10	35	116	82	0.3	0.002	0.13	0.004	1	11
Yogurt with whole milk, aroma and sugar	117	11	45	173	89.7	0.41	0.002	0.05	0.007	2.2	
Stirred yogurt with whole milk	160	15.5	68	220	95	0.42	0.002	0.1	0.009	1.4	15
Yogurt with whole milk	126	12.6	45.4	186	100	0.42	0.002	0.08	0.009	1.4	20
Cheeses											
Pyrénées cheese with cow milk	635	27	824	68	450	3.8	0.06	0.22	0.07	5.1	35
Blue cheese with cow milk	524	21.5	955	165	330	3.6	0.022	0.22	0.06	4.5	27
Feta cheese with cow milk	578	20	1428	145	352	2.55	0.05	0.34	0.06	6.5	12
Vacherin Mont d'or	175	0.06	0.6	0.2	1.5		12	0.1	0.3		0.2
Tilsit cheese whole milk	700	65	753	65	500	3.5	0.013	0.23	0.026	14.5	
Ricotta cheese with whole milk	207	11	84	105	158	1.6	0.006	0.38	0.021	14.5	
Edam	731	30	965	188	536	3.75	0.011	0.44	0.036	14.5	
Feta	493	19	1116	62	337	2.977	0.028	0.67	0.032	15	
Camembert with raw milk	235	15	802	150	286	3.78			0.21		
Brie	184	20	629	152	188	2.38	0.034	0.5	0.019	14.5	
Bleus	528	23	1395	256	387	2.66	0.009	0.31	0.04	14.5	
Colby	685	26	604	127	457	3.07	0.012	0.76	0.042	14.5	
Carvi	673	22	690	93	490	2.94	0.021	0.64	0.024	14.5	
Brick	674	24	560	136	451	2.6	0.012	0.43	0.024	14.5	
Romano	1064	41	1200	86	760	2.58	0.02	0.77	0.03	14.5	
Cheddar	721	28	621	98	512	3.11	0.01	0.68	0.031	13.9	
Fontina	550	14	800	64	346	3.5	0.014	0.23	0.025	14.5	
Gruyere	1011	36	336	81	605	3.9	0.017	0.17	0.032	14.5	
Muenster	717	27	628	134	468	2.81	0.008	0.41	0.031	14.5	
Gietost	400	70	600	1409	444	1.14	0.04	0.52	0.08	14.5	
Monterey	746	27	536	81	444	3	0.011	0.72	0.032	14.5	
Cheshire	643	21	700	95	464	279	0.012	0.21	0.042	14.5	
Camembert	388	20	842	187	347	2.38	0.038	0.33	0.021	14.5	
Provolone	756	28	876	138	496	3.23	0.01	0.52	0.026	14.5	
Cheeses with cream	80	6	296	119	104	0.54	0.004	1.2	0.002	2.4	
Neufchatel	75	8	399	114	136	0.52	0.004	0.28	0.016	3	
Port Salut	650	24	534	136	360	2.6	0.011	0.43	0.022	14.5	
Processed cheese with Cheddar	552	27	1489	169	513	2.84	0.008	0.19	0.016	14.4	
Switzerland Emmental	791	38	192	77	567	4.36	0.005	0.2	0.004	18.2	
Cottage cheese 1%	61	5	418.5	86	134	0.39	0.003	0.144	0.028	9	
Cottage cheese 2%	69	6	406+	96	151	0.42	0.003	0.16	0.028	10.2	

(*Continued*)

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Table 9.2. (*Continued*)

	Ca	Mg	Na	K	P	Zn	Mn	Fe	Cu	Se	I
Parmesan	1184	44	1602	92	694	2.75	0.02	0.82	0.032	22.5	
Limburger	497	21	800	128	393	2.1	0.038	0.13	0.021	14.5	
Queso Anejo (Mexico)	680	28	1131	87	444	2.94	0.037	0.47	0.008	14.5	
Mozarella without fat	961	33	743	106	656	3.94		0.31	0.034	18.9	
Mozarella	575	21	415	75	412	2.46	0.009	0.2	0.022	16.1	
Mexican cheese Queso Asadero	661	26	655	86	443	3.02	0.036	0.51	0.026	14.5	
Hard cooked cheeses	1050	43.8	405	103	690	5.2	0.05	0.4	0.13	8.2	30
Bleu de Bresse	450	17	602	118	320	2.5	0.02	1	0.1	16.1	48
Bleu d'Auvergne	563	18.1	113.6	84.8	301	2.68	0.02	0.3	0.07	3.71	27.2
Fresh cheese 0%	118	11.7	42.1	124	102	0.49	0.005	0.16	0.01	1.9	10
Processed cheese (25% fat)	346	24	282	152	990	8	0.02	0.3	0.5	7.4	20
Fresh cheese (type Petit suisse 20%)	117	10	34	98	125	0.48	0.06	0.2	0.01	2	14
Soft cheese with washed crust	329	15.2	617	90	359	6.2	0.02	0.15	0.07	5.8	31.5
Pont L'Evêque	485	20	690	128	431	5.1		0.37	0.05	4.8	35
Livarot	620	21.3	729	112	427	5.8	0.05	0.37	0.11	8.7	37
Beaufort	995	39.5	628	117	728	5.18	0.03	0.24	0.09	7.22	37.6
Fourme d'Ambert	490	20.5	1203	131	841	6.2	0.02	0.36	0.11	3.7	27
Maroilles	350	40	937	130	320	9	0.02	0.4	0.07	7.36	23.8
Morbier	760	30	990	100	520	7	0.04	0.3	0.06	8	20
Raclette	664	14	650	112	450	3.5	0.04	0.23	0.06	8	20
Reblochon	514	25.1	555	152	339	8.5		0.32	0.11	5.1	20
Saint Marcellin	138	20.2	1009	187	442	0.85	0.02	2.74	0.07	0.51	28.5
Saint Nectaire	539	30.4	477	98	304	5.3	0.035	0.22	0.07	5.1	32.1
Saint Maure	151	16.2	829	132	233	0.64		0.2	0.1	10.5	45.3
Saint Paulin	741	31.6	750	87.9	401	4.81	0.05	0.24	0.09	8.9	21.2
Tome	626	24.9	807	90	460	3.76	0.02	0.2	0.05	5	20
Cancoillotte	144	8	502	31	425	1.4	0.02	0.13	0.1	4.5	20
Cantal, Salers, Laguiole	798	27.5	875	125	494	3.8	0.04	0.42	0.09	5.4	21.8
Comté	909	48.9	412	118	664	5.1	0.03	0.49	0.1	7	24.4
Mimolette	860	37.1	1110	103	508	3.1	0.03	0.56	0.07	4.9	15
Maasdam	495		370	100	470	3	0.04	0.12	0.06	8	20
Lactic goat cheese with pasteurized or raw milk (type buchette)	120	15.1	624	228	203	0.585		0.273	0.05	10	30.6
Fresh goat cheese (type Palet or Crottin)	91.6	12.1	297		153	0.367		0.271	0.1	8	29.2
Lactic goat cheese with raw milk (type Crottin de Chavignole, Pico)	122	16.2	504		208	0.555		0.308	0.1	10	30.9

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Fresh goat cheese with pasteurized or raw milk (type Crottin or buchette)	95	11.7	369		158	0.4	0.1	0.28	0.1	8	29
Goat cheese with pasteurized or raw milk (type buche)	113	12.5	666	200	188	0.61		0.21	0.1	10	30
Roquefort	662	30	1809	91	392	2.08	0.03	0.56	0.034	14.5	
Hard cheese with sheep milk	722		738		498						
Pyrénées cheese with sheep milk	721	30	740	55	499			0.3			
Sheep cheese with flowered crust	486		676		200						

*Concentrations are expressed in mg/100 g of product except for Se and I expressed in µg/100 g.
Source: based on data from http://www.composition-des-aliments.fr/.

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Ca content varies in the following increasing order: milks/fermented milks/fresh cheeses < soft cheeses < semi-hard cheeses < hard cheeses. The concentrations can be higher than 1000 mg per 100 g of some cheeses like Parmesan (Tunick, 1987). The locations and forms of Ca depend on the type of dairy products. Thus, in acidified dairy products like fermented milks with pH close to 4.6, Ca is mainly in the ionic form (Ca^{2+}). In ripened cheeses (Camembert, Cheddar, and Emmental), Ca can exist in different chemical and physical forms of precipitates of Ca phosphate, Ca lactate, and Ca carbonate (Brooker *et al*., 1975; Metche & Fanni, 1978; Bottazzi *et al*., 1982; Le Graët *et al*., 1983, 1986; Karahadian & Lindsay, 1987; Le Graët & Brulé, 1988; Morris *et al*., 1988; Hannon *et al*., 2009). The presence of phosphopeptides and free fatty acids has also been determined in ripened cheeses and these molecules can bind Ca to form caseinophosphopeptide–Ca complexes and insoluble soap, respectively.

9.2.1.3 Dairy contribution to the total Ca intake and Ca absorption

The recommended dietary allowance for Ca is about 800–900 mg for adults, 1000 mg for pregnant and lactating women, and 1200 mg for adolescents. The food sources of Ca are multiple (Fishbein, 2004) and thanks to their high Ca contents, milk and dairy products are considered as one of the best sources. Their contribution to the total daily Ca intake is estimated at about three-quarters in the developed Western countries. For example, 600 mL of milk (or two glasses) provides two-thirds of the human recommended daily allowance. Among the dairy products, the best sources are the hard cheeses (Table 9.2).

Ca is absorbed via active transport and passive diffusion across the intestinal mucosa. One of the most important factors in Ca absorption is vitamin D, operating principally via its most active metabolite, 1,25-dihydroxyvitamin D (also known as calcitriol), and its intestinal receptor. Other substances in the diet influence the absorption of Ca. Protein, caseinophosphopeptides, lactose, and acidity are potential enhancers of Ca absorption in the intestine (Bennet *et al*., 2000; Tsuchita *et al*., 2001; Camara-Martos & Amaro-Lopez, 2002; Mora-Gutierrez *et al*., 2007a, b). Phytates, oxalates, long-chain saturated fatty acids, uronic acids, and fibers such as cellulose or hemicellulose can form insoluble complexes with Ca and decrease its absorption (Hansen *et al*., 1996). About two-thirds of Ca in the diet is not absorbed and consequently excreted in the feces.

Milk processing can modify the Ca content but also its nature and consequently its absorption. Smith *et al*. (1985) reported Ca from milk and yogurt was similarly absorbed. Recently, Seiquer *et al*. (2010) reported that heat treatment of milk may negatively affect the Ca availability. They concluded that consumption of a diet based on overheated milk led to significant reductions in apparent Ca absorption. The Ca in cheese is considered as highly absorbable, which is related to the presence of caseinophosphopeptides produced by proteolysis during ripening. Buchowski and Miller (1990) reported that Ca absorption averaged 76.8% from ripening Cheddar cheese and was not affected by the time of ripening; they also showed that absorption from $CaCl_2$, $CaCO_3$, fresh milk, milk at pH 5.35, and the cheeses was similar.

9.2.1.4 Physiological roles of Ca from milk and dairy products

As dairy products are excellent sources of Ca, research has elucidated the physiological roles of this ion. Thus dairy Ca plays different and multiple positive roles in bone health, weight management and obesity, colorectal and prostate cancers, blood pressure, and dental health. The most significant publications describing the impact of Ca from dairy products on human nutrition and health are discussed here (Guéguen & Pointillart, 2000; Pointillart & Guéguen, 2004; Barba & Russo, 2006).

9.2.1.4.1 Bone health/osteoporosis

The major part of the Ca (99%) and P (85%) in the human organism is located in the bone tissues. Ca and P are associated to form amorphous tricalcium phosphate and crystallized hydroxyapatite. Significant decreases in bone mass with increased susceptibility to fracture can be observed in cases of osteoporosis. The reasons for this disorder are complex, with genetic, hormonal, environmental, and nutritional factors all contributing. Among these, there are deficiencies in Ca and/or vitamin D in the diet. Consumption of milk and dairy products is a possible solution to preventing these deficiencies. Research at molecular and cellular levels, and *in vivo* with animals or humans, has documented the effects of dairy Ca on bone health. Many epidemiological studies on dairy calcium have been performed to test the effects on populations, because osteoporosis is a world public health problem. The results are sometimes confusing but today it is acknowledged that milk and dairy products contribute to better bone health (Renner, 1994; Heaney, 2000; Merrilees *et al*., 2000; Lau *et al*., 2001; Black *et al*., 2002; Cashman, 2002, 2006; Kato *et al*., 2002; Flynn, 2003; Lanham-New *et al*., 2007). Heaney (2000) concluded from 139 studies that there was a good relation between bone health and the consumption of Ca-rich foods. In 2005, Lanou *et al*. reviewed 58 studies on the effects of dairy products and

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total dietary Ca on bone integrity in children and young adults to assess whether evidence supports currently recommended Ca intake levels and the suggestion that dairy products are better for promoting bone integrity than other Ca-containing food sources or supplements. Eleven of the studies did not control weight, pubertal status, and exercise and were excluded. Ten studies were randomized controlled trials of supplemental Ca, nine of which showed modest positive benefits on bone mineralization in children and adolescents. Of the remaining 37 studies of dairy or not-supplemented dietary Ca intake, 27 found no relationship between dairy or dietary Ca intake and measures of bone health. In the remaining nine reports the effects on bone health were small and three were confounded by vitamin D intake from milk fortified with vitamin D. More recently, Huncharek *et al*. (2008) in a meta-analysis evaluating the impact of dairy products and dietary Ca on bone mineral content in children reported that increased dietary Ca/dairy products, with or without vitamin D, significantly increased total body and lumbar spine bone mineral content in children with low baseline intakes. It was also reported that different factors can promote this effect on bone health, these factors including caseinophosphopeptide (Matsui *et al*., 1994), milk basic protein (Yamamura *et al*., 1999; Toba *et al*., 2001; Aoe *et al*., 2005; Uenishi *et al*., 2007), and intervention of insulin-like growth factor (IGF)-I. This effect may be mediated by increased synthesis of IGF-I in growing bone (Kelly *et al*., 2003).

9.2.1.4.2 Weight management and obesity

The number of publications concerning this is increasing from year to year (Zemel, 2002, 2003, 2004, 2005, 2009; Barr, 2003; Novotny *et al*., 2004; Barba *et al*., 2005; Harvey-Berino *et al*., 2005; Zemel *et al*., 2005a, b; Barba & Russo, 2006; Murakami *et al*., 2006; Major *et al*., 2008; Bouglé *et al*., 2009; Shahar *et al*., 2010; Dougkas *et al*., 2011). Globally, a relation between increased dairy product consumption (or Ca intakes) and a reduction in body weight or body fat seems to exist. The physiological and molecular mechanisms underlying the impact of dairy constituents on adiposity are incompletely understood but may include effects on lipolysis, lipogenesis, and fatty acid absorption (Dougkas *et al*., 2011). According to Zemel (2002, 2004), an anti-obesity effect of dietary Ca and dairy foods is evident from animal studies, observational and population studies, and clinical trials. Dietary Ca exerts this effect on weight through the calcitrophic hormones, parathyroid hormone and 1,25-dihydroxyvitamin D. These hormones have been shown to respond to low-Ca diets and exert coordinated regulatory effects on human adipocyte lipogenic and lipolytic systems. In his review of 2004, Zemel reported that dietary Ca plays a pivotal role in the regulation of energy metabolism. It is explained that high-Ca diets attenuate adipocyte lipid accretion and weight gain during the overconsumption of an energy-dense diet, increase lipolysis, and preserve thermogenesis during caloric restriction, which thereby markedly accelerates weight loss. One the other hand, high-Ca diets have been shown to increase fecal fat excretion. Thus Christensen *et al*. (2009) in a meta-analysis of randomized controlled trials showed that dietary Ca has the potential to increase fecal fat excretion to an extent that could be relevant for prevention of weight (re-)gain. A similar conclusion was reported by Bendsen *et al*. (2008). The increase in fecal fat excretion could be due to Ca soap formation and/or binding of bile acids in the intestine. On the other hand, a recent study published by Lorenzen and Astrup (2011) showed that the nutrient combination of Ca and milk fat present in dairy products may play a key role in reducing fat absorption and may have the ability to maintain good cholesterol (high-density lipoprotein) while minimizing any increase in bad cholesterol (low-density lipoprotein).

9.2.1.4.3 Prostate cancer

Results of work studying the possible relationship between dairy product consumption and this type of cancer are conflicting and not clear. For Chan and Giovannucci (2001) and Chan *et al*. (2001), there is reasonable evidence that both vitamin D metabolites and Ca, and specifically Ca from dairy sources, play important roles in the development of prostate cancer. For Ahn *et al*. (2007), higher dairy product and dietary Ca intakes are modestly related to increased risk for prostate cancer. Parodi (2009) indicated that some epidemiological studies have shown an association between consumption of dairy products and Ca and risk of prostate cancer. Tseng *et al*. (2005) indicated that dairy consumption may increase prostate cancer risk through a calcium-related pathway. Among the explanations, Ca could depress the production of calcitriol, which has antiproliferative properties. Newmark and Heaney (2010) suggested that the high dietary phosphate of dairy products may more readily explain this risk rather than the increased Ca.

9.2.1.4.4 Colorectal cancer

This cancer is the third most common cancer worldwide and dairy products could reduce the risk of this type of cancer (Martinez & Willett, 1998; Norat & Riboli, 2003; Cho *et al*., 2004; Kesse *et al*., 2005; Larsson *et al*., 2006). For example, McCullough *et al*. (2003) indicated that

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the risk of colorectal cancer is reduced by 15–40% with a Ca intake higher than 1 g/day. Flood *et al*. (2005) showed also that Ca from diet and supplements is associated with a reduced risk of colorectal cancer in a prospective cohort study of women. Park *et al*. (2009) suggested that Ca intake is associated with a lower risk of total cancer and cancers of the digestive system, especially colorectal cancer. The binding of Ca to bile acids and fatty acids can reduce the proliferative stimulus of these organic compounds.

9.2.1.4.5 Hypertension

The inverse relationship between intake of dairy products and blood pressure levels was first suggested by several epidemiologic surveys in the early 1980s, which revealed low Ca intake in populations with increased prevalence of hypertension. Other clinical investigations provided further evidence of the beneficial association between Ca and lower blood pressure (Miller *et al*., 2000; McCarron & Reusser, 2002; Ruidavets *et al*., 2006).

9.2.1.4.6 Oral health

Moynihan *et al*. (1999) reported that cooked cheese-containing meals increased plaque Ca concentration. One possible reason for this protective effect is that Ca exists in a readily available form for diffusion into the dental plaque. Thus it acts by preventing demineralization or by promoting the remineralization of enamel. It was also reported that caseinophosphopeptides in the presence of amorphous Ca phosphate can decrease and prevent caries (Reynolds, 1993) by reduction in demineralization and/or an increase in remineralization of enamel. Another effect of cheese on dental health can be related to the high buffering capacity of cheese, which is rich in Ca and P, reducing the decrease of dental plaque pH (Jenkins & Ferguson, 1966; Walther *et al*., 2008).

9.2.1.5 Calcium supplementation of dairy products

For the dairy industry, it is appealing to enrich milk with calcium to improve the functional, technological, and nutritional properties of milk. Several Ca salts are utilized for this supplementation, among them salts of gluconate, lactate, citrate, chloride, glycerophosphate, malate, phosphate, pyrophosphate, sulfate, and hydroxide and oxide (Weaver, 1998; Augustin & William, 2002; Ibeagha-Awemu *et al*., 2009). The most used Ca salts for enrichment of milk, yogurt, and cheese are gluconate, lactate, citrate, chloride, carbonate, and phosphate (Philippe *et al*., 2003, 2004, 2005). Citrate, carbonate, and phosphate Ca salts are weakly soluble in milk and can have undesirable organoleptic properties. Lactate and gluconate salts have good solubilities and bioavailabilities. The modifications of the physicochemical characteristics of Ca-supplemented milks, as with Ca chloride, are relatively well understood, and the changes in distribution of Ca and phosphate, caseins and water between the aqueous and micellar phases have been described (Philippe *et al*., 2003, 2004, 2005), as has a decrease in the heat stability of enriched milk. However, a positive impact of Ca enrichment of dairy products at the nutritional level has not been clearly demonstrated.

9.2.2 Phosphorus (P)

9.2.2.1 Phosphorus in the human organism and biological roles

The human organism has a reserve of about 800 g of P and 85% of this element is associated with Ca in bone tissue and teeth. The remaining 15% is located in the tissues in different molecular forms such as sugar phosphate, nucleotides and nucleic acids, phospholipids in cell membranes, and phosphoproteins. It plays an important role in the synthesis of adenosine triphosphate, the energy source for many cellular reactions. It participates in the regulation of enzyme activity through phosphorylation–dephosphorylation reactions. P in the form of inorganic phosphate, due to its acid–base properties, also plays an important role in maintaining the pH of extracellular fluids.

9.2.2.2 Contents and chemical forms of P in milk and dairy products

P is an important element in milk and dairy products (Table 9.2). In milk, its concentration expressed in total P is about 950 mg/L. In terms of chemical forms, its description is complex because different forms of P exist in organic and inorganic P. In organic P, the phosphate is bound to organic molecules such as casein (phosphorylation of serine to form phosphoseryl residues), phospholipids, RNA, DNA, nucleosides, nucleotides, and sugar phosphate. The phosphate ion can be differently ionized depending on the pH. At the pH of milk (about 6.7), there is an acid–base equilibrium between the forms HPO_4^{2-} and $H_2PO_4^-$, adding to the complexity of the different forms of P found in different locations. In milk, organic P is mainly associated with casein molecules, which are in the micellar phase. The other forms of organic P (phospholipids, nucleosides, nucleotides, sugar phosphate) are in the aqueous phase.

Inorganic P is distributed between aqueous and micellar phases and contributes to the mineral equilibrium of milk. At pH 6.7, inorganic P is located 50% in the aqueous phase and 50% in the micellar phase, forming nanoclusters of Ca phosphate. During the transformation of milk into dairy products, inorganic P is transferred to the aqueous phase, especially during acidification (Gaucheron, 2004; Gaucheron *et al*.,

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2004; Mekmene *et al*., 2010). Consequently, it is lost when the whey is removed during cheesemaking. This explains the different levels of P determined in different dairy products (Table 9.2). Processed cheeses contain important amounts of P because melting salts corresponding to polyphosphates are added during the process. During the ripening of soft (Camembert) and hard (Beaufort) cheeses, as described for Ca, inorganic P migrates from the interior to the surface of cheese (Le Graët *et al*., 1983, 1986; Le Graët & Brulé, 1988), inducing P heterogeneity between the surface and the center of cheeses. In the rind of some cheeses like Camembert, inorganic P precipitates with Ca to form insoluble Ca phosphate salts (Brooker, 1987; Karahadian & Lindsay, 1987; Hannon *et al*., 2009). Proteolysis during cheese ripening also induces the formation of phosphopeptides from casein molecules, which have the property to bind Ca and other cations (Vegarud *et al*., 2000).

9.2.2.3 Dairy contribution to the total P intake and P absorption

The current recommendation in the Western world is about 700 mg/day for adults and a normal diet provides about 800–1400 mg/day of P; 60–80% is absorbed in the small intestine mainly by intercellular passive transport. This absorption depends on the average concentration of phosphate in the intestinal lumen. When the intake of phosphate is reduced, active transport stimulated by calcitriol is also involved. There is no deficiency for this element because it is found in different foods and especially in milk and dairy products, which are good sources of P. For example, 600 mL (or two glasses) of milk provide about 75% of the recommended daily allowance. It is noteworthy that P can inhibit the absorption of other minerals (Ca, Mg, Fe) by combining with them to form poorly soluble salts.

9.2.3 Magnesium (Mg)

9.2.3.1 Magnesium in the human organism and biological roles

Magnesium is the fourth most abundant cation in the body after Na, K, and Ca and the second most important intracellular cation. The body of a 70-kg adult contains 15–20 g of Mg (Table 9.1). About 50% of Mg is located in the bone tissue with Ca and P. The rest of Mg is intracellular. The biological roles of Mg are multiple and complex. It is involved in cellular energy metabolism, activation of certain enzymes (phosphatase and kinase), DNA replication and transcription, translation of mRNA, synthesis of protein and glycogen, stabilization of cell membranes, blood clotting by inhibiting platelet aggregation, nervous conduction, neuromuscular transmission, ion transport, and activity of Ca channels.

9.2.3.2 Contents and chemical forms of Mg in milk and dairy products

In comparison with Ca, Mg is not abundant in milk and dairy products (Table 9.2). Its concentration in milk is about 120 mg/L. As noted for Ca, 99% of this ion is located in the skim milk. It is distributed between micellar (50 mg/L) and aqueous (70 mg/L) phases. This ion is associated with inorganic phosphate and citrate in the aqueous phase and in the nanoclusters of casein micelles. This distribution is sensitive to the physicochemical conditions, especially acid pH. Thus, during milk acidification, micellar Mg is solubilized in the aqueous phase of acidified milk (Gaucheron, 2004; Gaucheron *et al*., 2004; Mekmene *et al*., 2010). In dairy products, the concentrations of Mg are variable depending on the manufacturing process. Cheeses and especially hard cheeses are considered good sources of Mg (Table 9.2). During the ripening of hard cheeses such as Beaufort, Mg migrates from the interior to the surface of the cheese (Le Graët *et al*., 1986), inducing heterogeneity of this element in the cheese.

9.2.3.3 Dairy contribution to the total Mg intake and Mg absorption

Human daily consumption provides 250–500 mg of Mg, which corresponds to the recommended daily intake. Despite their relatively low Mg concentrations, dairy products are considered good sources of this ion. Two glasses (600 mL) of milk provide 65 mg, corresponding to about 16% of the human recommended daily allowance. Concerning its absorption, 30–50% of this element is absorbed in the jejunum and ileum. Lonnerdal *et al*. (1993) reported that Mg in human milk, cow milk, and infant formula was similarly absorbed and retained. This absorption is mainly performed by paracellular passive transport. Secondary saturable active transport may also be involved. As for Ca, excessive intakes of protein, phytates, soluble fiber (pectin type), oxalic acid or P slightly decreased Mg absorption, probably by forming poorly soluble complexes which are not well absorbed. Mg is in competition with Ca for intestinal absorption and the optimal Ca/Mg ratio is 2.

9.2.4 Sodium (Na), chloride (Cl), and potassium (K)

9.2.4.1 Sodium, chloride, and potassium in the human organism and biological roles

The total amount of Na in the body of a 70-kg adult is about 100 g (Table 9.1). Sodium is the principal cation of extracellular fluids. Most of the Na is contained in blood plasma. Sodium regulates the distribution of body water, water movement in the body, and exchanges between intracellular and extracellular water. Because of its high concentration in plasma, it contributes to plasma osmolality

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(300 mosmol/L) and consequently to blood pressure regulation. Na plays essential roles in nervous transmission and muscle contraction. On the other hand, Na modulates the activity of certain enzymes.

Like Na, Cl is mainly extracellular. The biological roles of Cl are relatively unknown, although it is the main counterion "neutralizing" Na and K. Increased concentration of Cl in cells increases cell polarization and decreases its excitability.

The amount of K in an adult body is 140–170 g, of which 98% is intracellular and 2% extracellular. The majority of K is contained in the cells of liver, muscle tissue (30–40% of total), kidney, bone, and red blood cells. The main biological functions are water balance, regulation of blood pressure, neurotransmission (involved in mechanisms of depolarization/repolarization), muscle contraction, and activation of enzymes involved in phosphorylation reactions.

9.2.4.2 Contents and chemical forms of Na, Cl, and K in milk and dairy products

Na and Cl contents in milk are about 450 and 1100 mg/L, respectively. These concentrations can be increased during mastitis. Na and Cl are mainly located in the aqueous phase of milk and dairy products, where they can be free or weakly associated with ions of opposite charge, as for Cl. In dairy products, especially cheeses and butter, the concentration of NaCl is increased by salting or brining. In cheese technology, this salt contributes to draining of the curd, organoleptic properties of the cheese, and selection of microorganisms and enzyme activities during ripening. The concentration of NaCl depends on cheese type. Some cheeses contain important amounts of NaCl, such as Roquefort blue-veined cheese (Table 9.2). Other cheeses do not contain supplemented NaCl, for example lactic cheeses (Table 9.1). Potassium content in milk is about 1500 mg/L (it is the most abundant mineral in cow milk). Like Na and Cl, it is mainly located in the aqueous phase of milk and dairy products. In these products, K can be free or weakly associated with ions of opposite charge. In cheese, its concentration is considered low (Table 9.2) because the major part of this ion is removed during curd draining in the whey.

9.2.4.3 Dairy contribution to the total Na, Cl, and K intakes and Na, Cl, and K absorptions

An adult organism requires 4–5 g/day of Na. Dietary intake of Na, mainly in the form of NaCl, is 1–4 g/day although for some it can be 8–15 g which is higher than the requirements. There is no deficiency of this element. Bioavailability of Na is complete and its absorption is not in competition with other nutrients. The intestinal absorption of Na, which takes place in the small intestine and secondarily in the colon, is very rapid thanks to transcellular and paracellular transports involving specific carriers. Intestinal absorption of Na is enhanced by the presence of glucose and amino acids, due to cotransport systems located mainly in the jejunum.

Like Na, Cl is almost completely absorbed from the gastrointestinal tract. Its mechanism of absorption is not well known. Dietary intake of NaCl is generally considered to be too high since it may increase blood pressure. For this reason, different research is in progress to reduce the quantity of NaCl in dairy products or replace NaCl partially by KCl. Milk and dairy products contribute about 10% of the total daily intake.

K needs are in the range 3–6 g/day depending on the physiological state. A normal diet provides 2–6 g/day of K and consequently deficiency of K is rare. Dairy products are excellent sources of K. Digestive absorption occurs in the gut and is rapid and almost complete. Different types of transcellular transport contribute to the absorption of K.

9.3 TRACE ELEMENTS IN MILK AND DAIRY PRODUCTS FROM THE COW

9.3.1 Iron (Fe)

9.3.1.1 Iron in the human organism and biological roles

The human body contains about 4 and 2.5 g of Fe in adult men and women, respectively, and it plays essential roles in many biological functions. It is involved in the formation of hemoglobin, myoglobin, and enzymes that play roles in many metabolic reactions. In the body, Fe exists in two forms: heme (70%) and nonheme (30%) (Tapiero *et al*., 2001; Coudray, 2004). Of total Fe, 30% is stored in the liver, spleen, and bone marrow. Deficiencies in Fe induce anemia with different metabolic perturbations such as reductions in physical and intellectual capacities, lower resistance to infections, and perturbations during gestation. Today, Fe deficiency is a public health problem worldwide, mainly concerning children, adolescents, pregnant women, and older people.

9.3.1.2 Contents and chemical forms of Fe in milk and dairy products

Milk and dairy products are poor in Fe (Table 9.2). In the literature, the reported concentrations in milk are variable probably due to possible contamination and analytical difficulties. Globally, the average content in milk is about 0.5 mg/L. In milk, Fe is not free but associated with other compounds like casein molecules, whey proteins, inorganic P, citrate, and lactoferrin (King *et al*., 1959;

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Hegenauer *et al*., 1979; Brulé & Fauquant, 1982; Fransson & Lonnerdal, 1983). Lactoferrin in cow milk is at low concentration (0.1 g/L) compared with human milk (1 g/L).

In most cheeses, the Fe concentration is less than 0.5 mg/100 g of product (Table 9.2). Hard cheeses are richer in Fe. During ripening of Beaufort cheese, Fe migrates from the interior to the surface of the cheese (Le Graët *et al*., 1986) inducing heterogeneity of this element in the cheese.

9.3.1.3 Dairy contribution to the total Fe intake and Fe absorption

The recommended daily allowance of Fe is 9 and 16 mg/day for adult men and women, respectively. As milk and dairy products have low concentrations of this element, their contributions to total Fe intake are low. Thus, 600 mL (two glasses) of milk provide only 0.1 mg, i.e., about 2.5% of the recommended daily allowance. Because of its high physiological importance, the bioavailability of Fe from milk has been extensively studied. It is reported that Fe bioavailability is greater than 50% for human milk and about 10% for cow milk. This difference may be due to the fact that Fe is mainly bound to lactoferrin in human milk and to casein in cow milk (Davidsson *et al*., 1994; Lonnerdal, 1997; Vegarud *et al*., 2000). The strong binding of Fe to casein does not favor good absorption. Gaucheron *et al*. (1997a, b) reported that the binding of Fe to casein is resistant to acid pH. Some compounds can influence the absorption of Fe positively or negatively. Vitamin C is recognized as a promoter of Fe absorption, while phytic acid is an inhibitor.

9.3.1.4 Iron supplementation of dairy products

Knowing that milk and dairy products are potential vectors for this trace element, several researchers have focused on Fe enrichment of dairy products (Gaucheron, 2000; Martınez-Navarrete *et al*., 2002; Juneja *et al*., 2004; Park, 2009). The ideal source of Fe would be one that had good bioavailability without changing the natural properties of milk, such as its nutritional value, sensorial properties, and resistance to the technological process. Such an Fe source does not exist for several reasons: (i) Fe is relatively reactive toward milk components, and the physicochemical and sensorial characteristics of milk and dairy products are sometimes altered; (ii) the different Fe sources on the market are relatively limited; (iii) the cost needs to be low to envisage distribution for large populations; and (iv) when added to milk or dairy products, Fe is not considered a natural element. Despite these difficulties, fortification of dairy products has been studied. Different milks (raw, pasteurized, whole, semi-skimmed and skim, with chocolate), different milk powders, whey proteins, caseins, fermented milks, cheeses, and infant formula have been enriched with different Fe sources (Gaucheron, 2000; Juneja *et al*., 2004; Park, 2009). At present, knowledge on enrichment of milk and dairy products is increasing but incomplete.

9.3.2 Copper (Cu)

9.3.2.1 Copper in the human organism and biological roles

The body of adult humans contains about 100 mg of Cu. Like the other minerals, Cu is essential since it contributes to different metabolic functions, for example Fe metabolism, formation of red cells, and maintenance of different organs. Copper is present in different metalloenzymes such as ceruloplasmin, cytochromes, amine oxidases, and superoxide dismutase. Its deficiency negatively influences blood, cardiovascular, connective tissue, bone, nervous and immune systems and contributes to osteoporosis, osteoarthritis, and cardiovascular problems (Coudray, 2004).

9.3.2.2 Contents and chemical forms of Cu in milk and dairy products

Milk and dairy products are poor in Cu (Table 9.2). The average Cu concentration in cow milk is about 0.1 mg/L. Reported high Cu contents are due to contamination caused by contact of milk or cheeses with equipment containing copper. Other reasons are rapid urbanization and industrial development causing environmental pollution. Thus, Simsek *et al*. (2000) reported abnormal levels of Cu (0.39, 0.58, and 0.96 mg/kg) in three different industrial regions in Turkey. In milk, the locations of Cu are multiple. In 1966, Samuelsson reported that natural Cu is about 15–20% associated with fat globules, about 35% with casein, and 28% with whey proteins. Aulakh and Stine (1971) showed by equilibrium dialysis that micellar casein, fat globule membrane proteins, α-casein, β-lactoglobulin, and β-casein were able to bind Cu. In whole milk, its distribution is as follows: lipids, 2%; whey proteins, 8%; casein molecules, 44%; and a low-molecular-weight fraction (inorganic P and citrate), 47%. Similar results were obtained by King *et al*. (1959), Martin *et al*. (1981) and Brulé and Fauquant (1982). Rafaowski and Zegarska (2006) indicated that the content of Cu in milk fat ranged from 0.010 to 0.089 mg/kg. It was shown that Cu can be associated with the milk fat globule membrane (O'Connor & O'Brien, 2006). The Cu-binding protein ceruloplasmin has also been detected in bovine milk (Hanson *et al*., 1967). The binding mode of Cu to casein is probably not only by electrostatic bonds. Gaucheron *et al*. (1997a) indicated that Cu is strongly bound to casein molecules

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and it was necessary to acidify at pH values less than 5 to disrupt the interaction between casein and Cu. The Cu concentrations in different dairy products are low (Table 9.2) and uncertain because the distribution of Cu in cheeses and fermented milks is not well documented. It is reported that Cu influences proteolytic enzymes and the activity of the microorganisms used in the manufacture of Swiss-type cheeses (Maurer *et al*., 1975a, b). On the other hand, Cu, which is a catalytic metal, can induce lipid oxidation.

9.3.2.3 Dairy contribution to the total Cu intake and Cu absorption

The human needs of Cu are not well identified. Some studies suggest 2–2.6 mg/day, while others indicate less than 2 mg/day. As they are poor in this element, milk and dairy products are not considered good sources; 600 mL of milk provides only 0.3 mg (5%) of the recommended daily allowance. The bioavailability of Cu from cow milk has not been studied in detail. Lonnerdal *et al*. (1985a) and Lonnerdal (1996, 1997) reported that the bioavailability of Cu from human milk is higher than that from cow milk and infant formula. As for Fe and Zn, a large proportion of Cu in cow milk is bound to casein, which does not promote its bioavailability (Lonnerdal, 1997; Vegarud *et al*., 2000). On the other hand, it is reported that proteins, amino acids, carbohydrates, and ascorbic acid promote Cu availability, whereas phytate, Zn, and Fe appear to have little influence on Cu absorption. Lactose may also interfere with Cu utilization in milk and overall diet. Strain (1988) postulated that the strong association between the consumption of milk and dairy products and the incidence of ischemic heart disease indicates a central role for dietary Cu deficiency in the etiology of this disease. It is noteworthy that early introduction of Cu-contaminated animal milk feeds possibly caused Indian childhood cirrhosis (Tanner *et al*., 1983).

9.3.3 Zinc (Zn)

9.3.3.1 Zinc in the human organism and biological roles

Zinc contributes to growth and development of the human organism. This element is found mainly in muscles (60%) and bones (30%). Zinc is involved in the activity of over 200 enzymes and hormones (Tapiero & Tew, 2003; Coudray, 2004). It participates in protein synthesis, activation of DNA and RNA polymerases, metabolism of fatty acids, and prostaglandin synthesis. It stabilizes the structure of peptide hormones such as insulin and thymulin. Zinc is also considered as an antioxidant. Deficiency of this trace element results in growth failure, impaired parturition, neuropathy, decreased and cyclic food intake, diarrhea, dermatitis, hair loss, bleeding tendency, hypotension, and hypothermia.

9.3.3.2 Contents and chemical forms of Zn in milk and dairy products

The concentration of Zn in cow milk is 3–5 mg/L; Anderson (1992) indicated a concentration of 3.96 mg/L. Nearly all (99%) of the Zn is present in the skim milk where it is associated with casein micelles (95%), probably in interactions with phosphoseryl residues of casein molecules and/or inorganic phosphate present in micellar and aqueous phases (Blakeborough *et al*., 1983). Zinc is also associated with citrate molecules in the aqueous phase (Singh *et al*., 1989; Pabon & Lonnerdal, 2000). During acidification, Zn is shifted from casein micelles to the aqueous phase (Gaucheron *et al*., 1997a). Cheeses are good sources of Zn (Table 9.2) with concentrations higher than 30 mg/kg for hard cheeses. The location of Zn in cheeses is not well established but during the ripening of Beaufort cheese, Le Graët *et al*. (1986) reported that it migrates from the interior to the surface of the cheese, inducing heterogeneity of this element in the cheese.

9.3.3.3 Dairy contribution to the total Zn intake and Zn absorption

Zn needs for adult humans are estimated to 12 and 14 mg/day for women and men, respectively. Milk and dairy products contribute significantly to these needs. Thus, 600 mL (two glasses) of milk provide 2.4 mg, corresponding to about 20% of the human recommended daily allowance. The average absorption of Zn is about 20%. A higher bioavailability has been reported for human milk compared with cow milk. This difference can be explained by the fact that Zn in human milk is bound to ligands of low molecular weight like citrate while more than 90% of Zn in cow milk is bound to casein (Lonnerdal, 2000; Pabon & Lonnerdal, 2000; Vegarud *et al*., 2000). Caseins and phytates limit the absorption of Zn. Bobilya *et al*. (1991) showed that in neonatal pigs the apparent Zn absorption was greater from nonfat dry milk (83%) and low-fat plain yogurt (87%) than from Zn carbonate (75%). The study of Rosado *et al*. (2005) suggests that milk and yogurt when added to a plant-based meal significantly increase Zn absorption but do not affect Fe absorption. The mechanism of Zn absorption is not well known and it seems that Zn uses an apical transporter common to other divalent cations like Fe (Conrad & Umbreit, 2000; Pérès *et al*., 2001).

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9.3.4 Selenium (Se)

9.3.4.1 Selenium in the human organism and biological roles

Selenium is a metalloid with physicochemical properties similar to those of sulfur. This trace element is a cofactor of enzymes and most biological functions of Se are related to its role in glutathione peroxidases. These antioxidant enzymes protect cell membranes against free radicals. The antioxidant role of Se against cardiovascular diseases and tissue aging is known (Brown & Arthur, 2001). Severe deficiency of Se promotes the development of certain cancers (Coudray, 2004; Navarro-Alarcón & Cabrera-Vique, 2008; Navas-Acien *et al*., 2008).

9.3.4.2 Contents and chemical forms of Se in milk and dairy products

Milk contains significant amounts of this element (Alaejos & Romero, 1995; Tinggi *et al*., 2001; Navarro-Alarcon & Cabrera-Vique, 2008; Navarro-Alarcon *et al*., 2011). A wide variation of Se levels is found in the literature and the average concentration is about 30 µg/L. This element is mainly located in the skim milk where it is associated with casein molecules and whey proteins (Van Dael *et al*., 1991). Deschuytere *et al*. (1987) indicated that Se was mainly bound to whey proteins. Knowledge of Se contents in dairy products, the possible chemical forms and association with other milk components is low. The concentration of Se in different dairy products is reported in Table 9.2. Foster *et al*. (1998) reported a reduction in intrinsic and fortified Se levels in cow milk after heat treatment (pasteurization, spray drying). Navarro-Alarcon *et al*. (2011) reported an Se concentration of about 30 ng per gram of cow milk fermented products.

9.3.4.3 Dairy contribution to the total Se intake

The dietary recommendation for Se differs between countries but is about 75 and 60 µg/day for men and women, respectively. The absorption of Se is relatively high (40–80%) and depends on its chemical form (Fairweather-Tait, 1997). Organic forms, generally linked to an amino acid, are better absorbed than selenite. Se is absorbed by an active transport mechanism similar to that of sulfate. The bioavailability of Se can be affected positively by thiols and vitamin C or negatively by heavy metals, P, and methionine. Because of its significant Se concentration, cow milk is considered an important source of this element. Thus, 600 mL (two glasses) of milk provide 20 µg of Se. Depending on the country, the contribution of dairy products to daily intake of Se is between 8 and 39% of the human recommended daily allowance (Flynn & Cashman, 1997). Shen *et al*. (1996) estimated by an *in vitro* method the Se bioavailability from human (11.1%), cow (6.8%), goat (6.2%), and sheep (<2%) milk. On the other hand, Se from milk and other sources is well absorbed in people with ileostomies (Chen *et al*., 2004).

9.3.4.4 Selenium supplementation of dairy products

Nutrition is the principal reason for enriching different dairy products with Se. Allen and Miller (1980) studied Se binding and distribution in goat and cow milk, and they indicated that these characteristics differed as a function of pH. In these milks, the added Se was mainly located in the aqueous phase. After acidification of the enriched milks, Se was shifted from the aqueous phase to the casein fraction, which is precipitated. Alzate *et al*. (2010) reported that enrichment of fermented milk with Se was possible without affecting the microbial development and the sensorial properties of the enriched products. It is also possible to increase the levels of Se by supplementing the dietary Se intakes of cows using a selenized yeast product (Muñiz-Naveiro *et al*., 2005). Recently, Hu *et al*. (2008) indicated that Se-enriched milk protein isolate is superior to selenized yeast in terms of its bioavailability and capacity to suppress oncogenesis.

9.3.5 The other trace elements in milk and dairy products from the cow

The literature on this subject is less abundant, and the results describing the content and chemical forms of these elements in milk and dairy products from the cow are sometimes in disagreement. On the other hand, as their concentrations are low, dairy contributions to total intake of these elements and their absorption from milk and dairy products have not been detailed and consequently are not well known.

9.3.5.1 Manganese (Mn)

Mn is a trace element present in bones, kidneys, liver and pancreas. It contributes to different biological functions such as blood coagulation, metabolism of sugar, absorption of Ca, and nervous functions. This ion participates in the activities of several enzymes, for example superoxide dismutase, pyruvate decarboxylase, and glycosyltransferases. Deficiency and toxicity of this element are rare. In cow milk, the Mn concentration is 20–50 µg/L. Concerning its distribution, Mn binds to the fat globule membrane and also to lactose synthase (Lonnerdal *et al*., 1981). Lonnerdal *et al*. (1985b) studied the Mn-binding proteins in cow milk and found that 32% of total Mn was in whey, 67% in casein, and 1% in lipid. In cow whey, Mn was mostly complexed to ligands with molecular masses less than 200 Da. The recommended daily allowance is about 2 mg. Milk and

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dairy products are considered as poor in this element. Cashman (2011b) reported that that absorption of Mn from cow milk is relatively low (2.4 ± 1.7%).

9.3.5.2 Iodine (I)

This element is a constituent of thyroxine (T_4) and triiodothyronine (T_3), which are important thyroid hormones that play roles in the growth and development of humans. Its deficiency causes a thyroid dysfunction named goiter, mental and psychomotor disorders, growth abnormalities, and infant mortality.

Milk and dairy products contain this element but the concentrations reported in the literature vary with the author and are not always in accordance. Indeed, the concentrations are functions of the animal feed, the season (higher concentrations are found in winter), and the exposure to iodophors and cleaning agents containing I. Pennington (1990) determined that the content of I in fluid milks averaged 23 µg/100 g, and ranged from 16 µg/100 g in the summer and fall to 34 µg/100 g in the winter in the eastern states of the USA. The concentration of this element was highest in the winter (27 µg/100 g) and lowest in the summer (19 µg/100 g) and higher in the western and central states (25 and 27 µg/100 g, respectively) than in the eastern states (18 µg/100 g). In 2003, Dahl *et al*. studied the I concentration in Norwegian milk and dairy products and indicated that low-fat milk from the summer season had significantly lower median I concentration (88, range 63–122 mg/L) compared with low-fat milk from the winter season (232, range 103–272 mg/L). The median I concentration of organic summer milk (60 mg/L) was significantly lower than the I concentration of organic winter milk (127 mg/L). Whey cheese (Tine Gudbrandsdalsost) I concentration was significantly higher (803 mg/kg) than the median I concentration in casein cheeses such as Jarlsberg and Norvegia (201 and 414 mg/kg, respectively). Cressey (2003) showed that the mean values for almost all the dairy products tested in New Zealand fell within a fourfold range (0.06–0.20 mg/kg). The sole exception was butter (all samples less than 0.04 mg/kg). Fresh milk products (standard and trim milk) sampled in winter (June 1997) had significantly higher I content than the products sampled in spring (October 1997). Also, fresh milk products from the south of the country had higher I content than samples from the north of the country, whereas samples from the north had significantly higher I contents than samples from the central region.

Pearce *et al*. (2004) evaluated the I concentration in different cow milk samples and found at least 88 µg per 250 mL, ranging from 88 to 168 µg (116.0 ± 22.1 µg per 250 mL). More recently, Soriguer *et al*. (2011) studied the I concentration in 362 samples of milk from 45 commercial brands and compared it with the milk I status in studies undertaken 17 years earlier. They found that mean concentrations of iodine in the milk rose from 1991 (117 ± 37 µg/L) to 2008 (259 ± 58 µg/L). The I concentration was greater in skim milk (273 ± 52 µg/L) than in semi-skimmed milk (254 ± 57 µg/L) or whole milk (251 ± 61 µg/L). The winter samples had a greater concentration of iodine (270 ± 55 µg/L) than the summer samples (247 ± 58 µg/L), independently of the type of milk.

Concerning the location of I in milk, Cashman (2011b) indicated that 80–90% of the I is in the inorganic form, mainly as iodide and is in the water-soluble fraction, while 5–13% is bound to proteins through either covalent bonds or loose physical associations, with less than 0.1% bound to fat (Miller *et al*., 1975).

The recommended daily allowance is 150 µg for both men and women. Milk and dairy products are considered as determinants of I intake (Girelli *et al*., 2004; Soriguer *et al*., 2011) but estimation of the dairy contribution to the total intake of I is not clear. It has been estimated as 53–61% of I intake in winter and 24–29% of intake in summer (Flynn & Cashman, 1997), or 37% of total intake in the UK but only 6–7% in Germany (Cashman, 2011b). The absorption of I is rapid and almost complete.

9.3.5.3 Fluoride (F)

The body contains approximately 2.5 g of F especially in bones and teeth. This element is essential for life but excess can induce fluorosis, a disease negatively affecting the health of bones and teeth. The mean concentration of F in milk is about 20 µg/L (range 10–140 µg/L). The concentrations can be increased by fluorine pollution. Garrec and Plebin (1986) reported that the total F content in milk from cows grazing on fluoride-contaminated pastures (0.26 µg/g) is increased compared with the content in milk from cows on a "normal" grass diet (0.11 µg/g). Among dairy products from contaminated milk studied by these authors, the F concentrations in cream and cheese are the highest. During processing, large amounts of F accumulate in these products, and the F concentrations of cream and cheese are multiplied three times (0.80 µg/g) compared with the concentration in milk. However, the study demonstrates that not only milk but also dairy products are poor sources of F even in cases in which the F content of the ambient air is notably high. Concerning the location of F in milk, Wheeler *et al*. (1988) reported that 46–64% of the F in milk is free and the remainder bound to proteins. Pasternak *et al*. (1998) indicated that F is mainly in free form and only a small fraction is bound to proteins. No data indicate clearly the dairy contribution to total F intake and F absorption from milk and dairy products.

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9.3.5.4 Chromium (Cr)

The biological role of Cr in humans is not clear and depends on its oxidation state. Cr^{3+} seems important and positive for life, especially in glucose metabolism, where it enhances insulin activity. Conversely, Cr^{6+} is considered toxic and a chemical carcinogen. The concentrations of Cr in cow milk are variable. Muzzarelli *et al*. (1983) reported a range between 0.2 and 3.6 µg/L with an average of 2 µg/L, whereas Renner (1983) reported a range of 5–50 µg/L with an average of 17 µg/L. Cocho *et al*. (1992) reported a concentration of 0.83 µg/L. Ambushe *et al*. (2009) indicated levels of total Cr ranging from 33.2 to 57.1 mg/L. On the basis of these results, milk samples contained 1.31–3.28% Cr(VI). This indicates the presence of Cr(VI) in relatively low concentrations with reference to total Cr. Milk and milk products are minor sources of this element (Larsen & Rasmussen, 1991).

9.3.5.5 Lead (Pb) and cadmium (Cd)

In general, milk and dairy products contain very low concentrations of these elements except when the dairy animals have consumed polluted water, fodders and feeds. With the aim of measuring the impact of this type of pollution on the quality of milk, different studies have been done in polluted areas. For example, Krelowska-Kulas (1990) reported that the Pb and Cd content of cow milk from four dairy centers located various distances from the iron and steel works "Lenin" in Nowa Huta, a part of Kraków, was about 10 times higher than in milk from an agricultural area far from industrial influences. Cabrera *et al*. (1995) reported concentrations of Pb and Cd from not detectable to 0.750 µg/g and 20.0 ng/g, respectively. Ayyadurai *et al*. (1998) studied the concentrations of Pb and Cd in the milk of cow and buffalo reared in the city of Chennai (Madras). The concentration of Pb ranged from not detectable to 36.6 ng/mL and from 4.0 to 25.2 ng/mL in cow and buffalo milks, respectively. The concentration of Cd in cow and buffalo milks was undetectable. Tahvonen and Kumpulainen (1995) reported mean Pb content of 1.7 µg/kg in milk, 17 µg/kg in Finnish cheese, and 17–60 µg/kg in imported cheese. Rodríguez Rodríguez *et al*. (1999) found mean Cd concentrations in raw cow milk of 4.88 mg/L (range 0.7–23.1 mg/L) and in pasteurized cow milk of 4.30 mg/L (range 3.4–5.9 mg/L). The Pb concentrations in raw cow milk were 14.82 mg/L (range 1.3–39.1 mg/L) and in pasteurized cow milk 10.25 mg/L (range 6.9–19.6 mg/L). Patra *et al*. (2008) indicated that high levels of Pb (0.85 ± 0.11 µg/mL) and Cd (0.23 ± 0.02 µg/mL) were recorded in lactating cows reared around a Pb–Zn smelter and steel manufacturing plant. They concluded that increased milk Pb or Cd levels as a result of natural exposure of lactating cows to these environmental toxicants significantly influences the trace mineral composition of milk and such alterations affect milk quality and nutritional values.In France, transfer of Pb and Cd from raw milk to Comté cheese were studied by Maas *et al*. (2011), who found that the concentrations of both elements was largely below those dangerous for consumers. The contribution of milk and dairy products to the total intake of Pb and Cd is toxicologically insignificant but it is noteworthy that Pb and Cd can be toxic by a cumulative effect.

9.3.5.6 Cobalt (Co)

Cobalt is essential and is a constituent of cobalamin (vitamin B_{12}), which originates exclusively from microbial synthesis in the rumen. The Co content in milk is about 0.5 µg/L (range 0.4–1.1 µg/L) (Cashman, 2011b).

9.3.5.7 Molybdenum (Mo)

The human body contains about 0.07 mg/kg of this element. It is present at high concentrations in the liver and kidneys. It is a structural part of several enzymes and acts as a cofactor, and is also present in dental enamel. The Mo content of milk is about 50 µg/L, where it is mainly bound to xanthine oxidase, an enzyme associated with the milk fat globule membrane. Anderson (1992) indicated a concentration of 0.022 mg/L. The recommended daily allowance for this element is between 34 and 45 µg for an adult organism. Milk contributes significantly (36%) to total Mo intake (Cashman, 2011b).

9.3.5.8 Arsenic (As)

This element is one of the most toxic metals. The chemical industries and tobacco smoke are sources of contamination. It is also present in the natural environment (air, soil, and water). Intoxication with As has been reported after consumption of contaminated drinking water over a long period. As can inhibit different enzymes essential to metabolism, leading to death from multiple organ failure. Thus, As affects mainly the skin, lungs, and gastrointestinal tract and can cause nervous disorders, deteriorated motor coordination, respiratory and kidney damage, and different cancers (skin, liver, bladder and lungs).

The content of As in milk and dairy products is very low and not well documented. The International Dairy Federation (1986) indicated that the permitted As level should be less than 10 ng/g. The literature reports many different concentrations: <4.85 ng/g (Byrne *et al*., 1987), 0.14–0.77 ng/g (Cervera *et al*., 1994), <0.9–27.4 ng/g (Rosas *et al*., 1999), and 37.9 ng/g (Licata *et al*., 2004). Cashman (2011b) reported an As concentration of 20–60 µg/L. Ulman *et al*. (1998) reported a concentration

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of 4.932 ± 0.38 µg/L in milk of 36 cows grazing on shoulder grass of highways with heavy traffic.

9.3.5.9 Nickel (Ni)

This element has a vital biological role for most organisms. It is essential for the structure and function of several enzymes such as urease and superoxide dismutases. However, at high concentration Ni can also be toxic, carcinogenic, and an inducer of slight allergies to severe skin diseases. Ni normally occurs at very low levels in the environment. Sources of contamination are polluted air, drinking water, tobacco smoke, and food.

The concentrations of Ni in milk and dairy products are very variable. Thus, Ellen *et al*. (1978) reported an Ni concentration of 50 µg/kg (range <10–130 µg/kg) and 270 µg/kg (range 220–340 µg/kg) in liquid dairy products and cheese, respectively. Amaro *et al*. (1998) reported a concentration of 15.0 ± 3.81 µg/kg, whereas Cashman (2011b) reported a concentration of 26 µg/L. Sanchez-Segarra *et al*. (1997) studied the effects of pasteurization, sterilization, and drying on the Ni content of milk in Spain and reported values of 19.0, 16.5, and 579 µg/kg for pasteurized milk, sterilized milk, and dried milk, respectively. They indicated that the Ni content in pasteurized milk and sterilized milk was similar to that of fresh milk. These authors speculated that the high concentration of Ni found in milk powder could be due to contamination by containers and equipment.

From nutritional point of view, milk contributes significantly to the intake of Ni (11%) (Cashman, 2011b). It is also reported that milk is an inhibitor of Ni absorption (Solomons *et al*., 1982).

9.3.5.10 Silicon (Si)

In the human body, Si is the most abundant trace element after Fe and Zn, with 1–2 g for an adult. It is a constitutive element of bone, connective tissue, cartilage, and skin (Jugdaohsingh, 2007). There are few publications relating the Si concentration in milk and dairy products. Archibald and Fenner (1957) found a concentration of 1.43 mg/L. Anderson (1992) indicated a concentration of 0.434 mg/L and, more recently, Jugdaohsingh (2007) reported a mean of 0.31 ± 0.21 mg/100 g with range between 0.07 and 0.47 mg/100 g.

9.3.5.11 Boron (B)

Data on the biological role of this element are relatively lacking. Boron seems essential for the growth of bones. No deficiency syndrome in human has been described. Anderson (1992) indicated a concentration of 0.333 mg/L in milk. López-García *et al*. (2009) determined mean B content as 0.2–0.3 µg/mL in skim or whole milk.

9.4 MINERALS IN MILK AND DAIRY PRODUCTS OF OTHER SPECIES

The mineral content of milk and dairy products of sheep, goat, buffalo, yak, camel, and mare are briefly described in this section and in Tables 9.3 and 9.4. The values for these minerals are not always well described in the literature and are sometimes variable and confusing from one study to another. Breed, season, stage of lactation, herd management, and health of animals are possible variables.

9.4.1 Sheep

Sheep milk is rich in fat, lactose, protein (especially caseins), and minerals such as Ca, Mg, and inorganic P (Table 9.3). Globally the ash content of this milk is higher than that of cow and goat milk. As for other milks, Ca, Mg, Na, K, P, Cl, and Zn are found in significant amounts in sheep milk (Table 9.3). Ca, Mg, and P are distributed between the aqueous and micellar phases while Na, K, and Cl are mainly present in the aqueous phase (Table 9.4). The trace elements are also present (Table 9.3). Van Dael *et al*. (1993) reported that Se was mainly (98% of the total concentration) in the skim milk. In the skim milk, Se is associated with the casein fraction (>68%). Approximately 11, 4, and 17% Se is removed by dialysis from skim milk, casein, and whey, respectively, indicating a major association of Se with milk proteins. With regard to heavy metals, Anastasio *et al*. (2006) studied their concentrations in sheep milk collected in two regions of southern Italy and reported concentrations of 0.14, 0.07, and 0.18 µg/g for Cr, Cd, and Pb, respectively.

9.4.2 Goat

Global composition in terms of protein, lipid, and lactose of goat milk is similar to cow milk. The total mineral content (or ashes) of goat milk varies from 0.70 to 0.85%. The composition is similar to that described for cow milk (Table 9.3). However, goat milk is distinguished by high Cl and K contents. The distribution of Ca, P, and Mg between the soluble and colloidal phases of milk are similar for cow and goat milks (Table 9.4). Compared with cow milk, the solubilization of micellar Ca and inorganic P induced during acidification of goat milk are very similar to that of cow milk (Vesperini-Jaubert, 1992). Goat milk also contains trace elements (Table 9.3). Swamy and Mathur (1983) reported the Fe content in milk from 22 Beetal goats as 1.35 ± 0.079 mg/L. Van Dael *et al*. (1992) indicated the presence of Se in goat milk, with a major part of this element (94% of the total) in the skim milk. These authors reported that Se in skim milk was mainly associated with the casein fraction (>69%). Approximately 9, 7, and 24% of Se is removed by dialysis from skim milk, casein, and

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Table 9.3. Average or range of total mineral contents present in milks of different species.

	Ca	P	Mg	K	Na	Cl	Fe	Cu	Mn	Zn	I	Se
Cow	110–125	95–120	9–13	140–160	50–60	100–110	0.04	0.01	0.02	0.5	0.021	0.96
Sheep[1]	193	158	18	136	44	160	0.08	0.04	0.007	0.57	0.020	1.00
Sheep[2]	195–200	124–158	18–21	136–140	44–58	110–112	0.072–0.122	0.04–0.068	0.053–0.009	0.52–0.75	0.0104	3.1
Sheep[3]	170	150	18	120	44	82	0.03	0.1	trace	0.7	ND	ND
Goat[1]	134	121	16	181	41	150	0.07	0.05	0.032	0.56	0.022	1.33
Goat[2]	126	97	13	190	38	160	0.055	0.03	0.008	0.34	0.008	2.0
Goat[3]	100	90	13	170	42	150	0.12	0.03	trace	0.5	0.007	1.0
Goat[4]	0.83 – 1.92		0.07 – 0.22	0.84 – 1.81	0.31 – 0.88	ND	0.30 – 1.28	0.06 – 0.33	ND	1.37 – 6.03	ND	3.4 – 22.7
Buffalo[5]	174	119	ND	64	ND	ND	301	ND	2.47	6488	ND	ND
Buffalo[6]	188	89	18	112	47	59	ND	ND	ND	ND	ND	ND
Buffalo[7]	112	99	8	92	35	ND	0.161	0.35	0.27	0.41		ND
Buffalo[8]	163–224	89–137	16–30	102–148	45–57	57–106	0.04–1.3	0.07–0.26	0.038–0.066	0.32–0.73	0.86–1.94	ND
Yak[9]	147.2	79.36	6.0	107.6	47.8	ND	ND	ND	ND	ND	ND	ND
Yak[10]	154.5.	92.2	15.41	ND	ND	ND	0.057	0.11	0.006	0.73	ND	ND
Camel[11]	114	ND	10.5	156	59	ND	0.29	ND	0.05	0.53	ND	ND
Camel[12]	76–196.5	49–148	4–20.9	60–211	36–90.2	ND	0–0.37	0.011–0.15	0.02–0.19	0.28–0.44	ND	ND
Mare[13]	132.7	88.4	10.2	66.5	19.8	ND	0.037	0.064	ND	ND	ND	ND
Mare[14]	66	21.4	3.7	46.4	13.	23.4	ND	ND	ND	ND	ND	ND
Mare[15]	82.29	49.88	6.587	51.72	16.66	ND	0.1209	0.0249	0.00544	0.199	ND	ND

All concentrations are expressed in mg/100 g except for Se (μg/100 mL). ND, not determined.

Sources: based on data from [1]Park *et al*. (2007); [2]Raynal-Ljutovac *et al*. (2008); [3]Tamime *et al*. (2011); [4]Herrera *et al*. (2006); [5]Benincasa *et al*. (2008); [6]Ahmad *et al*. (2008); [7]Patiño *et al*. (2007); [8]Pandya & Khan (2006); [9]Silk *et al*. (2006); [10]Li *et al*. (2011); [11]Al haj & Al Kanhal (2010); [12]El-Agamy (2006); [13]Schryver *et al*. (1986b); [14]Holt & Jenness (1984); [15]Csapó-Kiss *et al*. (1995).

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Table 9.4. Total (T) and soluble (S) concentrations (mmol/L) of ions in milk of different species.

	Ca		Mg		Na		K		P_i		Cl	
	T	S	T	S	T	S	T	S	T	S	T	S
Sheep[1]	56.8	5.2	9.0	2.9	20.5	20.5	31.7	31.7	39.7	11.7	17.0	17.0
Goat[1]	23.1	8.0	5.0	3.4	20.5	20.5	46.6	46.6	15.6	9.0	34.2	34.2
Mare[1]	16.5	6.4	1.6	1.3	5.7	5.7	11.9	11.9	6.7	4.1	6.6	6.6
Buffalo[2]	47.1	8.2	7.3	3.5	20.3	18.4	28.7	26.0	27.7	9.2	16.6	16.3

P_i, inorganic phosphate.
Sources: based on data from [1]Holt & Jenness (1984); [2]Ahmad *et al*. (2008).

whey, respectively, indicating a major association of this element with milk proteins. Recently, Navarro-Alarcon *et al*. (2011) reported levels of Se and Zn in eight commercial goat milk fermented products of 28.32 ± 15.80 ng/g (range 7.81–57.5 ng/g) and 4.46 ± 0.932 µg/g (range 3.69–6.62 µg/g). Concentration of Se in different dairy products is also reported in Table 9.2.

From a nutritional point of view, goat milk seems to present some advantages. Thus, Shen *et al*. (1995) showed *in vitro* that bioavailability of Ca from goat milk is similar to that of $CaCl_2$ and thus is as high as in cow milk. On the other hand, Park *et al*. (1986) indicated a higher iron bioavailability in goat milk than in cow milk when fed to anemic rats. Barrionuevo *et al*. (2002) showed a beneficial effect of goat milk, with respect to cow milk, on the metabolism of Fe and Cu in control rats, especially those with malabsorption syndrome.

9.4.3 Buffalo

Compared with cow milk, buffalo milk is richer in fat, lactose, protein (especially caseins), and minerals such as Ca, Mg, and inorganic P (Table 9.3). The concentrations reported are also very different from one study to another. The influence of breed, year, season, and lactation stage on buffalo milk mineral content was reported by Patiño *et al*. (2007) and explain this large range.

Globally, the total and colloidal Ca and inorganic P concentrations are higher in buffalo milk than in cow milk (Ganguli, 1973; Sindhu & Roy, 1976; Varindra *et al*., 1994; Ranjan *et al*., 2005; Ahmad *et al*., 2008). Ahmad *et al*. (2008) showed that total and soluble concentrations of Mg and Na were higher in buffalo milk than in cow milk, whereas total and diffusible concentrations of K and Cl were higher in cow milk than buffalo milk (Table 9.4). Compared with cow milk, solubilization of micellar Ca and inorganic P during acidification of buffalo milk was quantitatively different (Ahmad *et al*., 2008).

9.4.4 Yak

The world production of yak milk is relatively small compared with cow milk and consequently research on this milk is very limited. The few results reported in the literature describe different compositional values. Globally, it is described as rich in protein and lipid. For the mineral fraction, Silk *et al*. (2006) and Li *et al*. (2011) indicated that the major minerals were more concentrated in yak milk compared with cow milk (Table 9.3). With regard to Cd and Cr, Sun *et al*. (2011) found, in fresh milk collected from 63 lactating yaks from Qinghai plateau in China, a Cd concentration of 0.305 ± 0.014 µg/g (range 0.278–0.341 µg/g) and a Cr concentration of 0.433 ± 0.028 µg/g (range 0.382–0.506 µg/g). In the same way, descriptions of the mineral composition of yak milk products are also limited, with some exceptions. Thus, the mineral composition of 32 samples of naturally fermented kurut from Qinghai in China was reported by Zhang *et al*. (2008). The concentrations were 140 ± 13.3 mg/100 mL, 146 ± 13.6 mg/100 mL, 1379 ± 23.7 mg/kg, 154 ± 16.5 mg/kg, 283 ± 18.9 mg/kg, and 5.74 ± 0.868 mg/kg for Ca, P, K, Mg, Na, and Zn, respectively.

9.4.5 Camel

The concentrations of the different minerals in camel milk are very variable and large (Table 9.3). Konuspayeva *et al*. (2010) indicated variations in the concentrations of Ca and P during lactation, with a maximum value (1.43 g/L for Ca and 1.16 g/L for P) at the beginning of lactation and minimal values (0.70 and 0.57 g/L, respectively) around the lactation peak. The means were respectively 1.05 and 0.83 g/L. Globally, the mineral concentrations of camel milk, especially Ca, Mg, P, Na, and K, are similar to those determined for cow milk (Table 9.3). It is often reported that camel milk is rich in Cl. In camel milk, Ca, Mg, and inorganic P are also distributed between the micellar and soluble phases. About two-thirds of Ca and Mg are

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associated with casein micelles. The calcium phosphate present in the casein micelles is solubilized during acidification (Attia *et al*., 2000). The ions K, Na, and Cl are mainly present in the aqueous phase of milk. With regard to the other trace elements, and as observed with the other milks, the values are variable. The variabilities observed are due to possible differences in breed, feeding, and analytical methods (Konuspayeva *et al*., 2009; Al haj & Al Kanhal, 2010). Konuspayeva *et al*. (2010) indicated that the Fe concentration varied from 1.00 to 2.67 mg/L with a mean of 1.73 mg/L. Konuspayeva *et al*. (2011) measured Cu, Zn, Pb, and Cd in raw and fermented camel milk (shubat) from different regions with a high risk of pollution (Almaty, Atyrau, Kyzylorda, Zhambyl and South Kazakhstan region). They found that the concentrations of these heavy metals were on average very low for Cu (<0.5 mg/L), normal for Zn (around 5 mg/L) and Cd, but slightly high for Pb.

9.4.6 Mare

Mare milk is less concentrated in the major compounds compared with cow milk. Mare milk is close to human milk, with low nitrogen content, a low casein-to-whey protein ratio, and high lactose content. The concentrations of macroelements, except K, are lower than in bovine, caprine, or ovine milk (Table 9.3). It is noteworthy that the mineral composition (Ca, Mg, P, Na, and K) decreases progressively during lactation (Schryver *et al*., 1986a; Csapó-Kiss *et al*., 1995; Summer *et al*., 2004). With regard to trace elements contained in this milk, Anderson (1992) reported concentrations of 0.097, 0.155, 0.224, 0.016, 0.014, 0.161, and 1.835 mg/L for B, Cu, Fe, Mo, Mn, Si, and Zn, respectively. As described for the other milks, one part of Ca and P of mare milk is bound to casein micelles and the other part is in the soluble phase (Table 9.4). Holt and Jenness (1984) showed that about 60 and 40% of total Ca and inorganic P are associated with the micellar phase. In the same study, these authors estimated ionic Ca in horse milk at 2.5 mmol/L. During acidification of mare milk, mineral solubilization is induced as observed with cow milk (Bornaz *et al*., 2010). However, the kinetics and the acid gel were described as very different from acid gel from cow milk.

9.5 CONCLUSION

Extensive studies have investigated the concentrations, forms, and locations of different minerals in different milk and dairy products. Today, milk and dairy products are considered important sources of minerals and there is no doubt about their nutritional significance. Despite this large body of knowledge, results in the domain of "nutrition health" are sometimes confusing and contradictory. This is because of different complexities:

- the complexity of milk and dairy products, where minerals exist in different chemical and physical forms;
- the complexity of the human diet, where the physicochemical conditions change and consequently the interactions described in milk and dairy products are modified;
- the complexity of digestion;
- the biological variability between species and between individuals of the same species;
- the biological complexity of humans, who exist under different physiological states.

A great many research projects are in progress in order to better understand these complexities in the future.

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10
Vitamins in Milks

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10.1 INTRODUCTION

Milk is an essential component in mammalian nutrition, being the sole source of nutrients for a determined period following birth. However, in adult human nutrition, milk and dairy products maintain a highly significant position in particular geographical areas of the world, depending on sociocultural behaviours and feeding habits. For example, milk consumption varies from about 180 kg of milk per capita per year in Iceland or Finland to 50 kg in the Far East in countries like China or Japan (Haug *et al*., 2007). The current general trend in Western countries is a reduction in the consumption of milk and dairy products as a consequence of the negative effects attributed to saturated fatty acids on heart disease and obesity. However, it should be emphasised that bovine milk is also an essential source of some micronutrients, especially vitamins such as retinol (vitamin A), riboflavin (vitamin B_2) and cobalamins (vitamin B_{12}) as suggested by the relative contribution of bovine milk in recommended dietary allowances (RDA) for humans (Table 10.1). Moreover, because milk contains an almost complete complement of vitamins, among other constituents, its contribution to the human diet could have benefits not only for health but also for metabolic regulation, with its impact depending on nutritional status (Smilowitz *et al*., 2005). Different studies combining micronutrient concentrations in food ingredients and the eating habits of populations in France (Etude Individuelle Nationale sur les Consommations Alimentaires INCA-2; Coudray, 2011), the Netherlands (Leyden Longevity Study and three Dutch Food Consumption Surveys; Vissers *et al*., 2011) and the USA (National Health and Nutrition Examination Survey; Drewnowski, 2011) agree that milk and dairy products are good sources and among the first contributors to vitamin A, D, B_2, B_5, B_9 and B_{12} intakes in human nutrition. The interest in milk and dairy product consumption is reinforced when considering their global nutritional density and, above all, their limited cost, which make them very attractive sources of vitamins (Drewnowski, 2011).

Vitamins are organic substances usually classified according to their solubility. Fat-soluble vitamins include vitamins A, D, E and K while the water-soluble vitamins comprise those belonging to the B-complex plus ascorbic acid (vitamin C). In general, under each micronutrient called 'vitamin' is a family of molecularly related compounds. It should be noted that at least one member of each vitamin family is usually found in milk, although sometimes at very low concentration. Differences in milk vitamin concentrations result from the combination of their specific origin (diet composition, microbial synthesis in the gastrointestinal tract, endogenous synthesis by animal tissues in some cases) that condition their availability for the dairy animal and their transfer to milk, and regulatory factors such as digestive processes, physiological status or health of the animal.

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Table 10.1. Contribution of bovine milk to the reference intake of vitamins.*

Vitamins	Mean concentration (µg/L)	Dietary reference intake (µg/day)		Contribution to RDI (%)	
		Min.	Max.	Min.	Max.
A†	385	600	900	11	16
D	10	5	15	17	50
E	700	11 000	15 000	1.2	1.6
K	3	60	120	0.6	1.3
B_1	420	900	1200	9	12
B_2	1650	900	1300	32	46
B_3‡	865	12 000	16 000	1.4	1.8
B_5	3430	4000	5000	17	21
B_6	390	1000	1700	6	10
B_9§	50	300	400	3	4
B_{12}	4	1.8	2.4	42	56
C	7500	45 000	90 000	2	4

*Values estimated for the consumption of a cup (250 mL) of bovine milk according to concentrations from USDA reference 01077, Milk, whole, 3.25% milkfat.

†Vitamin A is expressed in retinol equivalent (RAE).

‡Vitamin B_3 is given in niacin equivalents (NE).

§Vitamin B_9 is given in dietary folate equivalents (DFE).

Historically, vitamins were discovered and studied as factors for which nutritional deficiencies caused specific diseases: vitamin C and scurvy, vitamin B_3 and pellagra, vitamin D and rickets. Today, the main diseases resulting from clinical vitamin deficiencies have been identified and characterised and RDAs have been proposed for each vitamin to give a reference dietary intake required to avoid clinical deficiency symptoms (Table 10.1). However, studies on the biological properties of vitamins and their mechanisms of action are still being investigated because of their potential link with chronic diseases such as osteoporosis, diabetes, obesity or certain forms of cancer. This hypothesis is sustained by results from observational studies, for example vitamin K intake has been associated with a lower risk of hip fracture (Shea & Booth, 2008). Consequently, dietary reference intakes may be viewed as minimal requirements because they only represent a recommendation estimated on the basis of avoiding clinical signs of deficiency. The SUVIMAX study reported that vitamin D status in 14% of healthy subjects in France did not meet the lowest recommended value (Chapuy *et al.*, 1997). Thus, dietary intakes of vitamins and the suggestion that food ingredients (such as milk and dairy products) should have an optimal nutritional value are still a question of interest. Improving the nutritional quality of milk can be achieved through natural means by optimisation of the diet given to dairy animals or through technological processes such as milk fortification. Both ways have been explored and could lead, in combination, to optimisation of the nutritional quality of milk. However, in this chapter we focus only on the nutritional and animal factors of variation, mainly in the dairy cow.

10.2 AVAILABILITY OF VITAMINS IN MILK IN RELATION TO HUMAN HEALTH

10.2.1 Fat-soluble vitamins

10.2.1.1 Vitamin A

Vitamin A comprises a group of several related molecules with a common molecular structure composed of two isoprene motifs and a terminal ring. The generic term 'vitamin A' is used for compounds containing a β-ionone moiety (others than carotenoids) with the biological activity of all-*trans* retinol. All-*trans* retinol (alcohol form, Fig. 10.1), 11-*cis* retinal (aldehyde form), retinoic acid (carboxylic acid form) and its esters are the most recognised members of the vitamin A family (Lidén & Eriksson, 2006). All-*trans* retinol is generally considered as the main form of the vitamin; it is also the sole vitamin A component that occurs naturally in bovine milk. However, provitamins A belonging to the carotenoid family are generally present in milk as a result of transfer from the cow's diet.

Carotenoids are liposoluble pigments found mainly in plants (and in microorganisms). The carotenoid family is composed of carotenes (among them, all-*trans* β-carotene) and xanthophylls (the oxidised forms). They are characterised

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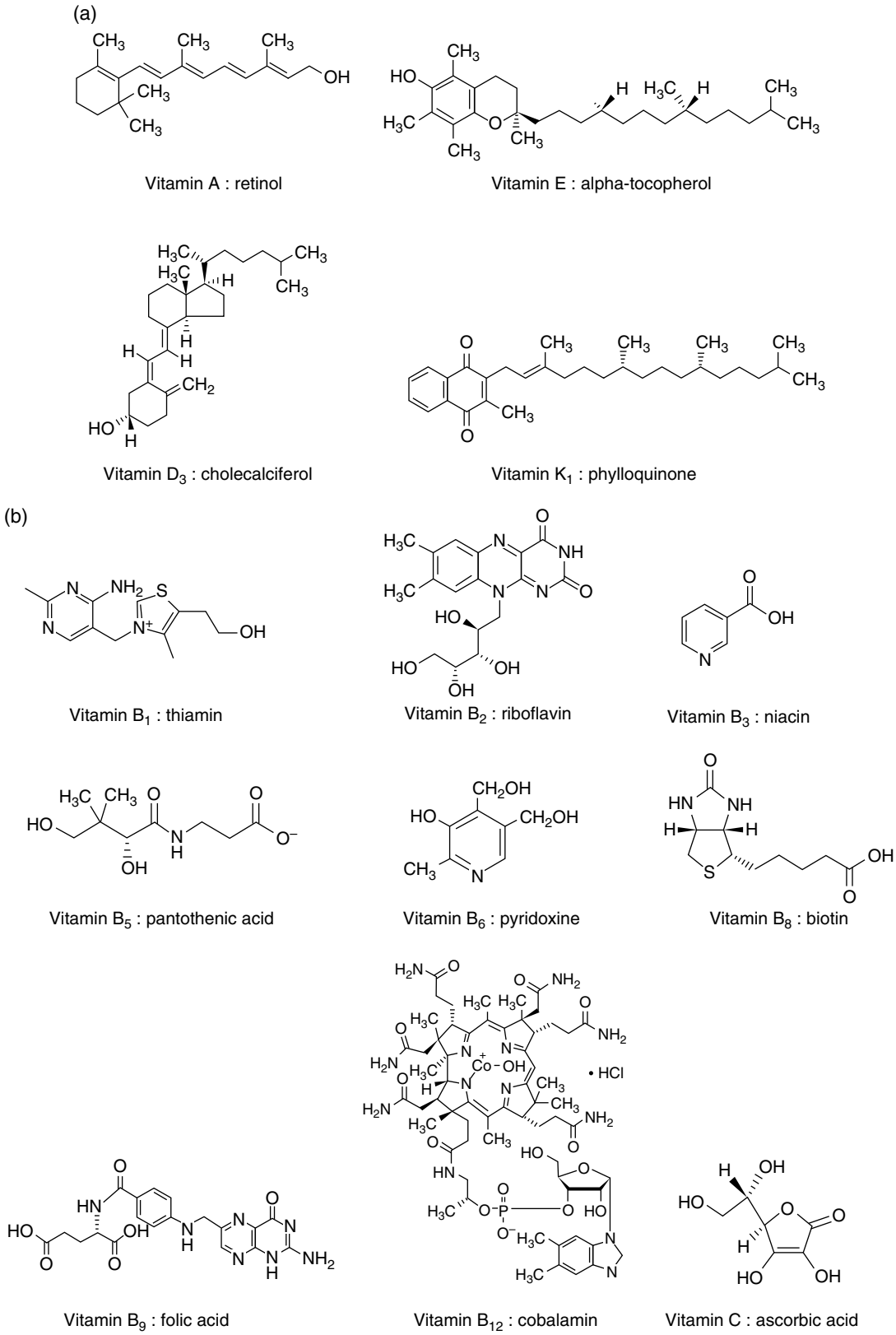


Figure 10.1. Fat-soluble (a) and water-soluble (b) vitamins in milk.

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by a linear polyisoprene structure with conjugated double bonds that can have a ring at one or both ends of the molecule (Nozière *et al*., 2006). Retinol and related compounds with vitamin A activity are synthesised by animal tissues from more than 50 carotenes. However, all-*trans* β-carotene possesses the highest vitamin A activity. Through symmetric cleavage in the enterocytes or in several other tissues such as liver, all-*trans* β-carotene is processed to two molecules of retinal by β-carotene 15,15′-monooxygenase activity and then reduced to retinol. All-*trans* β-carotene is also the main carotenoid found in bovine milk but is almost lacking in ovine milk due to its highly efficient intestinal conversion into retinal (Nozière *et al*., 2006). Total milk vitamin A activity is generally calculated as the sum of retinol and provitamin A carotenes.

Oils from fish liver (especially halibut, shark or cod) are by far the richest sources of vitamin A, although they do not play a great part in human nutrition. Products from ruminants are among the major sources of retinol in human diets, beef or sheep liver providing 150 μg/g and milk fat 4–14 μg/g; egg yolk contains around 4–9 μg/g (European Food Safety Authority, 2008). Other foods, such as meat, kidneys or fish, are not significant sources of vitamin A. In industrialised countries, half of the daily intake comes from preformed vitamin A, whereas the other half comes from carotenoids in foods from plant origin (FAO/WHO, 1988; European Food Safety Authority, 2008). Recommended dietary allowances of vitamin A for adults are 600 and 900 μg retinol equivalent per day for men and 500 and 700 μg per day for women (Table 10.1), according to FAO/WHO data (1988) and the US National Academy of Sciences (Dietary Reference Intakes, 2001), respectively. Higher intakes are recommended for pregnant (600–770 μg/day) or lactating (800–1300 μg/day) women. The relative participation of milk and dairy products in the daily intake of preformed vitamin A varies according to nutritional habits, especially liver consumption. In Europe, for example, liver is the major source of vitamin A (60–80%) in France, Greece, Italy and Spain whereas dairy products and milk provide 45–60% of the vitamin intake in Germany, Netherlands, Norway and Sweden (European Food Safety Authority, 2008).

Because of its numerous functions, vitamin A has been extensively studied and is currently the vitamin for which most is known about the cellular and molecular pathways. In the eyes, as 11-*cis* retinal, vitamin A is combined into different forms of opsin in the retina to allow vision in darkness, evaluation of brightness, and the differentiation of blue, red and green colours. In other tissues, mainly as retinoic acid (all-*trans* and 9-*cis* isomers), the mode of action of vitamin A is similar to that of hormones (such as steroid or thyroid hormones). Indeed, retinoid functions are mainly carried on through nuclear receptor pathways (RARs and RXRs). Several hundreds of genes participating in cell differentiation, embryogenesis, immune function, reproduction or growth, nervous system regulation (dopaminergic signalling) and intercellular communication are regulated by retinoids.

The first effect of vitamin A deprivation is xerophthalmia characterised by dryness of the eye epithelium and degradation of dark vision. Other symptoms of prolonged vitamin A deprivation are retarded growth, anaemia (likely through an effect on iron availability) and a decrease in reproductive efficiency that affects both males and females. In 1995, the World Health Organization played a major role performing a worldwide review on the global prevalence of vitamin A deficiency (World Health Organization, 1995). It was the most complete study performed on this topic. According to these data, in 1994, 251 million children (0–4 years old) suffered from vitamin A deprivation at a subclinical level.

Conversely, it should be noted that vitamin A is one of the vitamins for which excess can cause hypervitaminosis, resulting in the settlement by the Scientific Committee on Food of a tolerable upper intake level of 3000 μg of retinol equivalent as preformed vitamin A per day (European Food Safety Authority, 2008). This excessive dietary consumption of preformed vitamin A, mostly due to high consumption of liver, sometimes combined with dietary supplements enriched with vitamin A, affects 1–6% of consumers in western European countries (European Food Safety Authority, 2008).

10.2.1.2 Vitamin D

The term 'vitamin D' covers approximately 30 compounds belonging to the calciferol family. The molecular skeleton possesses a sterol structure with a central hydrophobic ring. The two precursor forms are ergocalciferol (vitamin D_2), synthesised in plants, and cholecalciferol (vitamin D_3; Fig. 10.1) produced in the skin of animals under the action of ultraviolet light on 7-dehydrocholesterol. Provitamin D has to follow a two-step sequence of hydroxylations to produce, first, 25-hydroxyvitamin D in the liver, then 1,25-dihydroxyvitamin D in kidneys (and to a lesser extent in several other tissues like brain, colon or prostate) (Holick & Chen, 2008). These latter dihydroxylated forms are the main biologically active molecules. Several other related metabolites have been characterised but they have a very low activity and a rapid clearance rate (DeLuca, 2004).

Like vitamin A, vitamin D is considered to have hormone-like activity, acting via a specific nuclear receptor to achieve its different functions. The 1,25-dihydroxyvitamin D produced by kidneys is secreted in plasma to reach the intestine and bone, its two main sites of action. The main role of vitamin D is to regulate calcium availability in the whole body. This is achieved through (i) stimulation of

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calcium absorption in the intestine, (ii) mobilisation of osteoclastic activity (calcium resorption) in bones, and (iii) calcium reabsorption at the kidney level (DeLuca, 2004). On the other hand, in other tissues, vitamin D is also considered a regulator of the expression of more than 200 genes implicated in cell growth, differentiation and immunity. Finally, 1,25-dihydroxyvitamin D_3 was also demonstrated to act on cancer cells by inhibiting their growth and inducing their maturation (Holick & Chen, 2008).

Because of its roles and the fact that it could be produced by the skin under the photolytic action of ultraviolet light, vitamin D should be considered a hormone. However, when sunlight-induced cutaneous synthesis of vitamin D is impaired, its dietary intake has to be sufficient to cover daily requirements and then it meets the definition of vitamin. In general, it is considered that vitamin D is poorly provided by food since it is usually not found in plants and is present in limited amounts in animal products (DeLuca, 2004), especially in products from animals raised under confined indoor housing conditions (Kurmann & Indyk, 1994). Vitamin D content in foods is currently of concern for the US Department of Agriculture (Holden *et al*., 2008), mostly because the available databases are rather poor. Moreover, analytical methods used to build these databases on food composition are not well defined and generally do not discriminate between D_2 or D_3 forms. The lack of reliable information on vitamin D activity in foods impairs calculations of dietary intakes and estimation of the relative importance of dairy products in human nutrition. Sea products (fish and shellfish) have the naturally highest vitamin D content, for example a portion of wild salmon can contain up to 1000 IU/100 g (Holick & Chen, 2008). By comparison, in bovine whole milk (3.25% fat, with added vitamin D), the mean vitamin D content is around 500 IU/L (13 µg/L; $N = 24$) according to United States Department of Agriculture (2012). In a previous version of the database, the proposed mean value obtained from a reduced sample set was 40 IU/L for 3.25% fat without added vitamin D. Several works have reported the relative vitamin D activity of each calciferol-derived compound. According to the authors, concentrations varied from 43–322 ng/L for vitamin D_3 to 145–685 ng/L for 25-hydroxyvitamin D_3, 4.2–5.4 ng/L for 1,25-dihydroxyvitamin D_3 and 27–45 ng/L for 24,25-dihydroxyvitamin D_3 when cows received between 4000 and 40 000 IU/day (Hollis *et al*., 1981; Reeve *et al*., 1982). The corresponding vitamin D activity was estimated to be 27–47 IU/L in bovine milk. At this level, without being fortified by vitamin D addition, milk could provide up to 50% of adequate intake (AI) to the consumer (Table 10.1; Dietary Reference Intakes, 1997; Weaver & Fleet, 2004; Holick & Chen, 2008). As a result of limited sun exposure and low dietary intake, one in seven adults (14%) was found vitamin D-deficient in the French study SUVIMAX, with a mean 3.4 µg ingested per day out of the recommended 10 µg (Chapuy *et al*., 1997). Moreover, several factors increase the risk of deficiency, such as ageing, season, increased skin pigmentation, sunscreen utilisation, obesity or several medications (Holick & Chen, 2008). The main consequences of vitamin D deficiency are growth retardation and rickets in children, increased osteopenia, osteoporosis and risk of bone failure, and muscle weakness in adults (Holick & Chen, 2008).

Given the prevalence of deficiency, hypervitaminosis is rare (Jones, 2008). Vitamin D toxicity is mostly due to excessive use of supplements because endogenous synthesis in the skin is closely regulated by needs and vitamin concentrations naturally present in foods are low.

10.2.1.3 Vitamin E

The small vitamin E family is composed of eight naturally occurring vitamers sharing a common structure: a chromanol ring with a phytyl C16 side chain (Fig. 10.1). For four of them, i.e. the tocopherols, the side chain is saturated whereas for the four others, i.e. the tocotrienols, three double bonds are spread along the side chain (Bjorneboe *et al*., 1990). For both groups, four different molecular forms exist (α, β, δ and γ), according to the number and position of methyl substitutions on the phenolic ring: three methyls for α, two methyls for γ and β and only one for δ-tocopherol and tocotrienol. Among the eight vitamers, α-tocopherol is the form most frequently present in nature; more precisely, α-tocopherol molecule contains three asymmetric carbons that lead to eight possible stereoisomers but the natural form is the RRR-α-tocopherol (Politis, 2012). It is the vitamer with the highest biological activity, the vitamin acting primarily as an antioxidant (Debier *et al*., 2005). In the molecule, the side chain allows efficient incorporation in biomembranes while the hydroxyl in the phenolic ring is the active site for free radical scavenging and protection of lipids from peroxidation (Bjorneboe *et al*., 1990). Vitamin E is the most important liposoluble antioxidant for human health. It protects polyunsaturated fatty acids from oxidation in cell membranes and in plasma lipoproteins. This is especially important in newborns for proper neural development and function but it also helps to prevent the development of degenerative diseases in adults (Bramley *et al*., 2000; Debier *et al*., 2005). Moreover, α-tocopherol participates in the maintenance of integrity of milk fat globule membranes (Baldi & Pinotti, 2008). Vitamin E also improves cellular immunity (neutrophils and macrophages) and prevents inflammatory conditions (Baldi, 2005).

The main vitamin E sources for human nutrition are plant oils (wheatgerm, sunflower seed, rapeseed, peanut, olive), cereals (wheat, barley), nuts (almonds), green vegetables

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(spinach, cabbage) and fruits (blackberries, tomatoes, avocado, blackcurrant) (Bramley *et al*., 2000). Animal products are relatively low in vitamin E and concentrations reported in bovine milk vary between 0.2 and 1.0 mg/L (Baldi, 2005; United States Department of Agriculture, 2012). The main form of vitamin E in cow milk is α-tocopherol (84–92%), the others being γ- tocopherol and α-tocotrienol (Baldi, 2005). Milk and dairy products represent only a minor part of the recommended daily intakes (11–15 mg/day for adults; Table 10.1; Dietary Reference Intakes, 2000) that otherwise are usually easily met through diet.

Experimental vitamin E deficiencies performed on laboratory animals have shown reduction in reproductive efficiency, muscular dystrophy, exudative diathesis, megaloblastosis, pulmonary degeneration, nephrosis and liver necrosis (Bjorneboe *et al*., 1990; Bramley *et al*., 2000). However, in humans, deficiencies are more often consequences of pathological situations such as lipid malabsorption syndromes like abetalipoproteinaemia (Bramley *et al*., 2000).

Very high doses of vitamin E are well tolerated. Toxicity has been mostly induced experimentally. At very high doses, vitamin E could impair functions of other fat-soluble vitamins, probably through a competitive effect on intestinal absorption.

10.2.1.4 Vitamin K

Vitamin K is the fourth and lesser known group of fat-soluble vitamins. Members of the vitamin K family share the same basic structure, i.e. a 2-methyl-1,4-naphthoquinone ring with a lateral carbon chain linked at the 3-position to the ring, but they differ in the structure of the side chain. Vitamin K_1, also called phylloquinone, possesses a C20 phytyl chain (Fig. 10.1), whereas in the group of vitamins K_2, the menaquinones (MK-*n*), the side chain is composed of a variable number (n=4–13) of isoprenyl units. Vitamins K_1 and K_2 have different biological origins since phylloquinone is the only form synthesised by plants and the menaquinones, with the exception of MK-4, are produced by bacteria in the digestive tract of human and animals (Van Winckel *et al*., 2009). Indeed, MK-4 synthesis is possible in mammal tissues from dietary phylloquinone or menadione (also called vitamin K_3). The latter is the synthetic form of the vitamin, used as a dietary supplement in animal husbandry. It could also be a metabolic intermediate in vitamin K metabolism in animal tissues (Okano *et al*., 2008).

Vitamin K was first discovered in the 1930s through its anti-haemorrhagic properties. It is now well known that vitamin K acts as a cofactor for the enzyme γ-glutamyl-carboxylase. This enzyme is responsible for the post-translational conversion of glutamate residues into γ-carboxyglutamates in some proteins during their secretion. These carboxyglutamate residues are calcium-binding groups, essential for the activity of the proteins in which they are found. It was considered first that vitamin K-dependent γ-carboxyglutamate proteins had restricted expression and distribution among tissues but it is now well known that vitamin K participates in haemostasis through coagulation factors II (prothrombin), VII, IX and X, and through proteins C and S (feedback mechanism) produced by the liver. This vitamin is also involved in calcium homeostasis (via osteocalcin and the matrix-Gla protein), inhibits apoptosis by regulating GAS-6 activity, and regulates signal transduction and growth development (Berkner, 2005; Van Winckel *et al*., 2009). Moreover, vitamins K_1 and MK-4 also have antioxidant properties, protecting cerebral development. Finally, MK-4 was also reported to have specific activities mediated by gene transcription regulation via the sterol and xenobiotic receptor SXR, such as the inhibition of tumour cell proliferation (Suhara *et al*., 2009).

Phylloquinone is the principal dietary form of vitamin K found mainly in green leafy vegetables (several hundred micrograms per 100 g) but also in non-leafy vegetables and in vegetal oils. Bovine milk contains low amounts of phylloquinone (0.6 µg/100 g), MK-4 and very low concentrations of other MKs. In dairy products, such as cheeses, significant concentrations of MK-8 and MK-9 are detected (5–10 µg/100 g and 10–20 µg/100 g, respectively) resulting from bacterial synthesis during the fermentation process (Shearer *et al*., 1996).

The RDA for vitamin K has been established at 1 µg/kg body weight per day on the basis on its anticoagulant activity. The resulting recommendations are 90 µg/day for women and 120 µg/day for men (Table 10.1; van Winckel *et al*., 2009). It seems these levels can be easily obtained through consumption of green leafy vegetables, but a study performed in the UK estimated the average daily intake at around 70 µg/day (Shearer & Bolton-Smith, 2000). The estimated mean availability of phylloquinone is close to 80%. However, it likely varies depending on the vegetal matrix, and the interaction with other dietary components, especially the presence of fat (Shearer *et al*., 1996; van Winckel *et al*., 2009). Moreover, 60–70% of the oral intake of phylloquinone is excreted within 3 days in urine (20%) and in faeces via biliary excretion (60–70%), in agreement with the rapid hepatic turnover of phylloquinone (Usui *et al*., 1990; Shearer *et al*., 1996). In conclusion, it implies that vitamin K has to be continuously provided to maintain tissue reserves and function of vitamin-K dependent proteins.

The most documented effect of vitamin K deficiency is haemorrhagic disease of the newborn (less than 6 months old), which can affect breast-fed babies, sometimes causing

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death, as placental transfer of the vitamin is limited and human milk is a poor source of the vitamin. Consequently, routine prophylaxis (oral or via intramuscular injection) with 1 mg vitamin K at birth has been adopted in most industrialised countries (Van Winckel *et al*., 2009). Elsewhere, vitamin K deficiencies leading to haemorrhagic risk in adults are rare (e.g. cases of fat malabsorption or cholestasis) but could easily be detected by the increase in plasma of proteins induced by the lack of vitamin K, such as under-γ-carboxylated osteocalcin or prothrombin. Much concern involves the link between vitamin K intake and skeletal health, especially with regard to reducing age-related bone loss. To date, the majority of the observational published studies have concluded that phylloquinone intake is negatively correlated with the risk of hip fracture. However, results of randomised controlled trials are more equivocal because phylloquinone supplementation does not usually lead to improvements in bone health in elderly people (Shea & Booth, 2008).

10.2.2 Water-soluble vitamins

10.2.2.1 B-complex vitamins

There are eight different families of B vitamins. The compounds are molecularly related within the family but not among them. B vitamins are water-soluble, can be produced by bacteria and, for most but not all of them, by plants (Roje, 2007). Their origin in milk is considered to be mostly from synthesis by rumen bacteria (National Research Council, 2001). Although B vitamins have different physicochemical properties and roles, most of them act as enzymatic cofactors involved in cellular metabolism.

10.2.2.1.1 Vitamin B_1

Vitamin B_1, also called thiamin or aneurin (Fig. 10.1), is synthesised by plants, yeast and bacteria (Roje, 2007). In cattle, the amount of thiamin synthesised daily by ruminal bacteria has been estimated to be between 28 and 72 mg/day, i.e. more than equivalent to the ingested fraction (Breves *et al*., 1981 cited by National Research Council, 2001). In the rumen, 48% of thiamin from plant origin is destroyed by microorganisms (National Research Council, 2001).

After absorption, thiamin is phosphorylated by enterocytes to its active form, the coenzyme thiamin pyrophosphate (or thiamin diphosphate). In virtually all cells, it is the cofactor of enzymes involved in several oxidative decarboxylations in cellular energy metabolism in the Krebs cycle (pyruvate dehydrogenase, α-ketoglutarate dehydrogenase) and in the conversion of branched-chain α-ketoacids (resulting from isoleucine, leucine and valine catabolism) to succinyl-coenzymeA or acetoacetate. Thiamin pyrophosphate is also the coenzyme of transketolase that participates in the pentose phosphate pathway (Depeint *et al*., 2006a).

In the human diet, thiamin is mostly found in cereals (especially wholegrain and fortified cereals) and pork meat (Allen, 2003). Mean thiamin concentration in whole cow milk is 0.46 mg/L (United States Department of Agriculture, 2012) and the average value in human milk is around 0.21 mg/L (Allen, 2003). The RDA for adults is 1.2 mg/day for males and 1.1 mg/day for females (Table 10.1; Dietary Reference Intakes, 1998). Consequently, milk is not an important source of thiamin in human nutrition. Thiamin intake in 6–8 month old, milk-fed children would not exceed 50% of their daily requirements (Lutter & Rivera, 2003). Babies and young breast-fed children are at-risk subjects among the population, since a reduction in thiamin dietary intake by the mother quickly results in infantile thiamin deficiency symptoms (Allen, 2003). Relatively recently (4 April to 13 July 2004), it led to a severe crisis in the overseas French area of Mayotte (located in the Comoros islands, between the east coast of Africa and Madagascar), where 32 babies were diagnosed with beriberi (62% lethality), the main disease resulting from thiamin deficiency (Institut National de Veille Sanitaire, 2004). Symptoms of clinical thiamin deficiency in children are peripheral neuropathy, encephalopathy and cardiac failure. Fortunately, such situations are limited today to undernourished women fed a polished rice-based diet or people in refugee camps and can be avoided by supplementations and prevention programmes (Allen, 2003). In adults, long-lasting thiamin deficiency induces first a loss of appetite until inanition leading to cardiovascular and neurological troubles. People with diseases related to food ingestion, with excessive alcohol consumption or living in nutritionally deprived conditions are considered at risk (Allen, 2003). Thiamin toxicity is very low.

10.2.2.1.2 Vitamin B_2

Vitamin B_2 is better known as riboflavin but is also referred to as lactoflavin, vitamin G or lactochrome (Fig. 10.1). Like thiamin, riboflavin is synthesised by plants and microorganisms. Mammalians are dependent on dietary intakes (Roje, 2007). In ruminants, dietary riboflavin is almost totally degraded by microorganisms (National Research Council, 2001). Consequently, riboflavin present in cow milk results from rumen synthesis.

After absorption in the proximal small intestine, riboflavin is activated in cells into flavin mononucleotide (FMN), then converted into flavin adenine dinucleotide (FAD). These forms are the main biologically active forms of vitamin B_2 (Powers, 2003). Both act as prosthetic groups of numerous enzymes (oxidases, reductases and dehydrogenases) called flavoproteins that are involved in

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oxidoreduction reactions essential for cell life (for review of an exhaustive list, see Depeint *et al*., 2006a). For example, FMN and FAD participate in electron transfer in the mitochondrial respiration pathway, in the initiation of fatty acid catabolism by β-oxidation, in the Krebs cycle, as cellular antioxidant protectants (by controlling glutathione reductase and glutathione peroxidase activities) and also in the metabolism of purine bases and amino acids (Depeint *et al*., 2006a).

In the human diet, most plant and animal products are sources of riboflavin. However, animal products like eggs, lean meat and milk are the major contributors to riboflavin intake (Allen, 2003; Depeint *et al*., 2006a). The riboflavin concentration in whole cow milk is 1.69 mg/L (United States Department of Agriculture, 2012) whereas the average value in human milk is considerably lower, around 0.35 mg/L in well-nourished women (Allen, 2003). The RDA for adults is 1.3 mg/day for men and 1.1 mg/day for women (Table 10.1; Dietary Reference Intakes, 1998). The calculated contribution of milk in the reference intake is between 60 and 80% (Haug *et al*., 2007). Thus, among water-soluble vitamins, riboflavin is one of the two B vitamins for which milk and dairy products are the greatest contributors to intake, especially in western countries (Powers, 2003). In developing countries, however, green vegetables are the greatest contributors (Allen, 2003). Riboflavin deficiencies are observed usually when dietary intake of animal products is low. It has a high prevalence among lactating women and the elderly in Guatemala, children in rural Mexico and in China (Allen, 2003; Powers, 2003). The corresponding disease is ariboflavinosis that has typical symptoms such as lip and tongue inflammation. However, due to its crucial role in cellular metabolism, numerous adverse effects occur following riboflavin deficiencies such as anaemia through interference with iron metabolism and possibly cancer, and cardiovascular diseases perhaps by interfering with folate-homocysteine metabolism (Powers, 2003; Depeint *et al*., 2006a). Vitamin B_2 hypervitaminosis has not been reported.

10.2.2.1.3 Vitamin B_3

The vitamin B_3 group, better known as niacin, is composed of pyridine-carboxylic acids and their derivatives (Fig. 10.1), mainly nicotinic acid and nicotinamide. This vitamin has also been called vitamin PP for 'pellagra preventive', because pellagra is a consequence of the deficiency. Both nicotinic acid and nicotinamide possess biological activity and at physiological doses have similar properties. At supraphysiological doses, however, their metabolism and properties are different.

Nicotinamide is the predominant form in foods but both vitamers could act as metabolic precursor of two major molecules playing vital metabolic roles, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). However, the pyridine ring of NAD^+ can also be synthesised *de novo* from tryptophan in animals and bacteria (Roje, 2007). As such niacin does not completely fulfil the definition of vitamin for many animal species, including human, because dietary needs are dependent on tryptophan supply. In the ruminant, it is likely that niacin produced by bacteria in the rumen covers the animal requirements and provides the most part of its secretion in milk, because the microbial degradation of niacin from feedstuffs seems extensive (Seck *et al*., 2010a).

In the intestine, both nicotinic acid and nicotinamide are absorbed and can be recovered in plasma, but they circulate mainly as NAD^+ as a result of their metabolism in tissue cells. NAD^+ and $NADP^+$ are cofactors in several hundred reactions, including mitochondrial respiration, glycolysis and β-oxidation. With riboflavin, niacin is one of the two B vitamins that are cofactors in oxidoreduction reactions. Among others, NAD^+ functions as an electron carrier for ATP production by cells. $NADP^+$ is a hydrogen donor for fatty acid, sterol or pentose biosynthesis, and also in the process of glutathione regeneration in the antioxidant protection of cells (Depeint *et al*., 2006a).

In human nutrition, the main sources of niacin are meat (above all liver, fish and poultry), cereal-based products including bread, milk and green leafy vegetables. However, availability of niacin in maize and some other cereals is low. In industrialised countries, cereals (fortified wholegrain or enriched) are the main sources of niacin (Allen, 2003). The reference value for preformed niacin in cow milk is 0.89 mg/L (United States Department of Agriculture, 2012), whereas the average concentration reported in human milk is 1.8 mg niacin equivalent per litre (considering that 60 mg of tryptophan allows the synthesis of 1 mg of niacin; Allen, 2003). The RDA value was set at 12–16 mg/day for males and 12–14 mg/day for females (from 9 to more than 70 years old; Table 10.1; Dietary Reference Intakes, 1998). Once the intakes are below the recommended values (rarely, except in cases of extreme alcoholism, anorexia, or seasonally in some at-risk countries), symptoms of deficiency occur: pellagra is a chronic wasting disease that was initially thought to be of infectious origin and which is characterised by dermatitis, dementia and diarrhoea. It is distinguished by a rash, pigmentation linked to exposure to sunlight, a red tongue, gastrointestinal disturbances and neurological abnormalities. However, at the beginning of the twentieth century, the link between pellagra and a diet based on maize, naturally poor in tryptophan and absorbable niacin, was established (Allen, 2003; Bogan & Brenner, 2008). Toxicity of niacin is low but at supraphysiological doses, side effects such as flushing are frequently reported whereas urticaria and gastrointestinal problems are less frequent.

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10.2.2.1.4 Vitamin B_5

Vitamin B_5 relates to only one compound, pantothenic acid (Fig. 10.1), and not to a family. Like the other B vitamins already presented in this chapter, vitamin B_5 can be synthesised by plants and microorganisms, but not by animals (Roje, 2007). In ruminant species, rumen microbial synthesis overcomes largely dietary intake; however, as the estimated degradation of the vitamin supplied in the diet is not complete (78%; National Research Council, 2001), it is likely that milk vitamin B_5 is a combination of dietary intake and rumen synthesis.

Vitamin B_5 is the precursor of the coenzyme A that plays a central role in numerous fundamental reactions: fatty acid oxidation, amino acid catabolism, acetylcholine synthesis, haem synthesis and as a prosthetic group in the Krebs cycle. It is also the precursor of the acyl carrier protein, essential to fatty acid synthesis. Consequently, in human diets, the vitamin is present in large amounts in foods, not only as pantothenic acid per se, but also in large amounts as coenzyme A. The latter is present mainly in animal organs, egg yolks, peanuts and beans, lean meats, milk (3.73 mg/L from United States Department of Agriculture, 2012; 2.8–4.2 mg/L from Souci *et al*., 2000 reported by Ragaller *et al*., 2011), potatoes and green leafy vegetables (Depeint *et al*., 2006a). Thus, a dietary deficiency in vitamin B_5 is extremely rare and it seems that it could only affect breast-fed babies of mothers fed a deficient diet (based mainly on refined cereals for example) but not healthy adults. The mean concentration reported in human milk is 2.2 mg/L (Allen, 2003) but with large variability (0.7–4.5 mg/L). Higher values have been observed (6.7 mg/L) in mothers with high intakes; moreover, a good correlation has been observed between pantothenic acid intake and its secretion in milk (Johnston *et al*., 1981). As a consequence of the absence of dietary deficiency in humans, there is no RDA value but only a proposed AI at 5 mg/day for normal adults (Table 10.1; Dietary Reference Intakes, 1998). Pantothenic acid is atoxic, even at doses 1000 times the AI.

10.2.2.1.5 Vitamin B_6

Vitamin B_6 is a little family of three compounds which differ by one group on the molecule (Fig. 10.1). This group may be an alcohol (pyridoxine), aldehyde (pyridoxal) or amine (pyridoxamine). All three are precursors of pyridoxal-5′-phosphate (P5P), a cofactor for numerous enzymes involved in amino acid metabolism. Among these are transaminases that participate in the catabolism of amino acids through the urea cycle, decarboxylases involved in haem synthesis, enzymes acting in cysteine, glycine or taurine synthesis, catabolism of serine leading to transfer of one-carbon units to tetrahydrofolate, and in tryptophan metabolism to NAD (Depeint *et al*., 2006b).

As reported for most other B vitamins, vitamin B_6 can be synthesised by plants and microorganisms but studies have indicated that net production in the rumen is limited (Santschi *et al*., 2005; Schwab *et al*., 2006). Consequently, vitamin B_6 secreted in cow milk would probably be mostly from dietary origin. Nevertheless, mean concentrations in milk seem rather stable at 0.36 mg/L (United States Department of Agriculture, 2012). In human milk, values are close to 0.13 mg/L but can decrease to less than 0.068 mg/L (Allen, 2003). It should be noted that pyridoxal is the major form of the vitamin B_6 in both cow and human milks (Vanderslice *et al*., 1983).

Human cells can synthesise P5P from the three vitamers but these vitamers must be provided by the diet. Despite the fact that they are not directly absorbable, dietary phosphorylated forms of the compounds are, by themselves, good sources of vitamin B_6 because the human intestine produces phosphatases that are able to hydrolyse them before absorption (Depeint *et al*., 2006b; Roje, 2007). In general, pyridoxine, pyridoxal and P5P are the major forms encountered in human diets. They are mainly present in vegetables, wholegrain cereals, nuts and muscle meats. However, the formation of a Schiff base between pyridoxal and lysine residues could occur during thermal processing or storage (depending on the maximal temperature) and limit vitamin B_6 availability.

As for pantothenic acid, clinical vitamin B_6 deficiencies are rarely encountered because of its widespread occurrence in foods. The clinical symptoms linked to vitamin B_6 deficiency are epileptic seizures, anaemia, renal failure and dermatitis (Depeint *et al*., 2006b). However, it was established that 10% of the US population ingests below half the recommended values (Depeint *et al*., 2006b) of 1.3–1.7 mg/day for adult males and 1.2–1.5 mg/day for females (Table 10.1; Dietary Reference Intakes, 1998). Chronic subclinical deficiency could affect the overall population in industrialised and developing countries and be associated with an increased risk of cardiovascular diseases, stroke, cancers (colon) and Alzheimer's disease (Depeint *et al*., 2006b; Roje, 2007). Acute toxicity of the vitamin is low but prolonged exposure to high doses could lead to neuropathy.

10.2.2.1.6 Vitamin B_8

Biotin (Fig. 10.1) is also called vitamin B_7, vitamin B_8, vitamin H or coenzyme R. It is synthesised by plants and bacteria from alanine and pimelic acid (Roje, 2007). Thus, biotin is usually produced by rumen microorganisms but the vitamin supplied by the diet is poorly degraded (National Research Council, 2001). Biotin is the prosthetic group of five cellular enzymes acting in the transfer of carboxyl groups. These enzymes include the two acetyl-coenzyme A

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carboxylases that participate in fatty acid synthesis, 3-methylcrotonyl-coenzyme A carboxylase that takes part in leucine catabolism, propionyl-coenzyme A carboxylase that participates in the catabolism of odd-chain number fatty acids and some amino acids before their entry into the Krebs cycle, and pyruvate carboxylase closely linked to glucose metabolism (Depeint *et al*., 2006a; Hassan & Zempleni, 2006; Roje, 2007). Independent to these metabolic activities, biotin plays a major role in the regulation of gene expression. Indeed, it is now well established that biotin is covalently bound to specific lysine residues in histones and would then regulate the expression of more than 2000 genes in human cells (Hassan & Zempleni, 2006).

Biotin deficiency is extremely rare in humans due to its high prevalence in food such as cow milk (8 µg/L, similar to human milk; Allen, 2003), liver, egg yolk, vegetables and fruits, and meat products. As is the case for pantothenic acid, some values of AI are proposed: 20–30 µg/day for adults, with the exception of lactating women (35 µg/day) (Dietary Reference Intakes, 1998). However, marginal biotin deficiency has been detected during pregnancy in 40% of women based on urinary excretion of 3-hydroxyisovaleric acid, which reflects methylcrotonyl-CoA carboxylase activity (Mock *et al*., 2002). Otherwise, the only reported clinical cases of dietary deficiency (neurological symptoms, hair loss and red facial rash among others) result from excessive consumption of raw egg white, which contains high levels of avidine, a natural ligand of biotin. Experimentally, it was demonstrated in laboratory animals that an inadequate biotin supply has teratogenic effects (Allen, 2003; Depeint *et al*., 2006a; Mock, 2009). No toxicity cases have been reported.

10.2.2.1.7 Vitamin B_9

Folates are a class of compounds with a chemical structure and biological activities similar to folic acid (pteroyl-L-glutamic acid; Fig. 10.1), the synthetic form of the vitamin. Folic acid is also the generic term for the vitamin. In nature, the molecule is present as dihydrofolate or tetrahydrofolate (THF), is substituted by different kinds of one-carbon units (methyl, formyl, methenyl, methylene and formimino), and possesses one (monoglutamate form) to seven glutamate residues in the side chain (polyglutamate form). Folates are synthesised by plants and microorganisms (Roje, 2007). In dairy cows, dietary folates seem to be largely destroyed in the rumen (National Research Council, 2001). It has been reported that, under some conditions, folic acid supplements increase milk production, suggesting that microbial synthesis in the rumen (16–21 mg/day; Santschi *et al*., 2005; Schwab *et al*., 2006) is not necessarily sufficient to cover cow requirements (Girard & Matte, 2005).

Folates are present in plasma as monoglutamylfolates, mostly 5-methyl-THF. However, the active form in cells is reduced and conjugated to a polyglutamate chain. Elongation of the glutamate chain requires first the demethylation of 5-methyl-THF by the vitamin B_{12}-dependent enzyme methionine synthase. Then, glutamate residues can be added to the side chain of the folates, which become active for transfer of one-carbon units in numerous reactions. The different forms of folates are essential for nucleic acid synthesis (purines, thymidylate, formyl-Met-tRNA) and in the methylation cycle that necessitates methionine regeneration from homocysteine before activation to *S*-adenosylmethionine, the primary methylating agent (for review, see Girard & Matte, 2005; Depeint *et al*., 2006b).

Main sources of folates in the human diet are green vegetables, grains, fruits, eggs and liver. Concentrations in bovine milk are 50–90 µg/L, mainly as 5-methyl-THF (Forssén *et al*., 2000; USDA Food Composition Data cited by Haug *et al*., 2007). The corresponding value in human milk is 85 µg/L (Allen, 2003). Recommended daily intakes of folates are 400 µg/day for both men and women but this level increases to 500 µg/day in lactating women and 600 µg/day during pregnancy (Table 10.1; Dietary Reference Intakes, 1998). On average, dairy products, including milk, could supply 10–15% of the daily intake in Western countries, especially among youth. In Western countries, it is now generally assumed that folate deficiency is the most prevalent vitamin deficiency. This is the reason why it was suggested in 1989 to increase the RDA from 200 to 400 µg/day (Bailey, 1995 cited by Forssén *et al*., 2000). Moreover, some countries such as the USA and the UK recommend daily supplements of folic acid for pregnant women (Forssén *et al*., 2000). The first symptom of folate deficiency is enlargement of erythrocytes and bone marrow cells as a result of the reduction in DNA, RNA and protein synthesis. This phenomenon is called megaloblastic anaemia. It can occur in cases of malnutrition, severe alcoholism or diseases that alter absorption efficacy (Forssén *et al*., 2000).

In pregnant women, the suboptimal status of the mother leads to premature birth, low birthweight, neural tube defects with spina bifida, and occasionally anencephaly. However, as this latter defect results from an early event in the development of embryos, folate supply should be sufficient even before conception. From conception, and throughout the pregnancy, folate requirements increase and consequently they are difficult to fulfil only by food. Under these conditions, folic acid supplementation reduces the risk of fetal development problems by 50–75% (Hathcock, 1997; Forssén *et al*., 2000). Folate deficiency also limits methionine regeneration, increasing plasma homocysteine concentration, which is an independent risk factor for

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coronary heart disease. Epidemiological studies have also shown a positive relation between high levels of folate intake and reduction in cancer risk (Forssén *et al*., 2000).

10.2.2.1.8 Vitamin B_{12}

Vitamin B_{12} is different to the other B vitamins: it is synthesised neither by plants nor animals. Indeed, only bacteria are able to produce cobalamins, which are a small group among the corrinoids. Corrinoids are cyclic molecules containing a core structure, the corrin part, which is identical to haem except for two features: the central metal ion is cobalt rather than iron, and one of the internal α-methene bridges is missing in the corrin nucleus (Herbert, 1988). Not all the corrinoids possess vitamin B_{12} biological activity in humans. Bacteria produce numerous corrinoids, vitamin B_{12} (cobalamins) and analogues, the latter being usable by microorganisms but not by mammals. Human tissues are only able to use specifically the cobalamins that are composed of the corrin ring plus an aminopropanol residue, a sugar, a nucleotide and an adduct linked to the cobalt atom (Fig. 10.1). The chemical nature of the adduct conditions the cobalamin to be biologically active in humans: hydroxocobalamin, aquacobalamin, 5′-desoxyadenosylcobalamin and methylcobalamin. Cyanocobalamin is the synthetic form of the vitamin and is not directly usable by human tissues. The cyanide group should be removed by a reductive decyanation reaction before its conversion to active coenzyme forms of the vitamin (Kim *et al*., 2008).

Vitamin B_{12} is absorbed in the ileum, so even if bacteria present in the human colon synthesise cobalamins biologically active for humans, these molecules are not absorbed (Herbert, 1988). Consequently, in the human, cobalamins are exclusively supplied by the diet, essentially from food ingredients of animal origin: milk and dairy products, meat, poultry, eggs, and fish. Bovine milk provides an average of 4.5 µg/L of vitamin B_{12} (United States Department of Agriculture, 2012). Adenosylcobalamin, hydroxocobalamin and methylcobalamin have been detected in milk (Farquharson & Adams, 1976; Fie *et al*., 1994) but hydroxocobalamin is the product of photolysis of the other cobalamins (Farquharson & Adams, 1976). The vitamin secreted in cow milk is synthesised by rumen microorganisms using dietary cobalt. It has been estimated that dairy cattle would require 0.34–0.68 µg cobalamin per kilogram liveweight and that rumen synthesis would cover the entire requirement (National Research Council, 2001). Recently, apparent ruminal synthesis of vitamin B_{12} was evaluated as 60–102 mg/day (Santschi *et al*., 2005; Schwab *et al*., 2006), which seems sufficient to cover cow requirements (estimated close to 442 µg for a 650-kg cow). However, efficiency of absorption in cows is low (Girard *et al*., 2001, 2009).

Human requirements have been estimated to be around 1 µg/day (Herbert, 1988; Depeint *et al*., 2006b) but the RDA is up to 2.4 µg/day in normal adults and 2.8 µg/day in lactating women (Dietary Reference Intakes, 1998). A glass (250 mL) of cow milk provides more than 1 µg (United States Department of Agriculture, 2012) which covers 42% of adult daily requirements for vitamin B_{12} (Table 10.1). Many epidemiological studies have reported a positive relationship between consumption of dairy products and vitamin B_{12} status (Miller *et al*., 1991; Tucker *et al*., 2000; Vogiatzoglou *et al*., 2009). These observations are sustained by results from a recent study, using pig as a model for intestinal absorption in humans, showing that vitamin B_{12} present in cow milk is substantially more available than the cyanocobalamin used in supplements (Matte *et al*., 2012).

Vitamin B_{12} participates in one-carbon transfer pathways since methylcobalamin is the cofactor of methionine synthase that catalyses the regeneration of methionine from homocysteine and 5-methyl-THF (vitamin B_9). This reaction is important for (i) the cellular methylation cycle, because the resulting methionine can be further activated into *S*-adenosylmethionine, the primary methyl donor of cells; (ii) the reduction in homocysteine levels, a risk factor for coronary heart diseases; and (iii) the transformation of methyl-THF into THF, the acceptor of one-carbon units for nucleic acid synthesis. Another role of vitamin B_{12}, as adenosylcobalamin, is to favour the catabolism of valine, isoleucine or odd-chain fatty acids into succinyl-coenzyme A by acting as a cofactor of the methylmalonyl-coenzyme A mutase. Cobalamin deficiency leads first to megaloblastic anaemia, described previously in the section on vitamin B_9. Because of their close metabolic interrelationships, a lack of vitamin B_{12} leads to secondary deficiency in vitamin B_9 (reduction in methionine availability, increase in homocysteine plasma concentration, hypomethylation including in DNA which could induce carcinogenesis). Moreover, it leads to accumulation of methylmalonic acid in cells and plasma that causes toxicity to mitochondria, hypoglycaemia, hyperglycinaemia and hyperammonaemia (Depeint *et al*., 2006b).

Clinical vitamin B_{12} deficiency affects only 1–2% of the elderly population in the USA (>60 years old), but 10–20% of this population has subclinical deficiency (Carmel & Sarrai, 2006). However, in most cases, vitamin B_{12} absorption is normal (Carmel, 2011). This observation is of importance because of the potential link between vitamin B_{12} and atherosclerosis risk. Deficiency in breast-fed children can also occur (slow growth and developmental delays) because human milk from normal well-nourished women is 10-fold less concentrated than bovine milk (0.42 µg/L; Allen, 2003). Deficiencies have been observed in several areas of the world

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(Latin America) but also in strict vegetarian populations in industrialised countries (Allen, 2003). There is no reported toxicity for this vitamin.

10.2.2.2 Vitamin C

Vitamin C is a small molecule with a lactone ring. It is present in tissues in its reduced (ascorbic acid) or oxidised (dehydroascorbic acid) forms (Fig. 10.1). Both forms have similar vitamin activity. The reversible conversion of the reduced form into its oxydised form explains its antioxidant property. Thus it acts as a cofactor for 11 enzymes that participate in collagen hydroxylation, biosynthesis of carnitine, catecholamines and norepinephrine, amidation of peptide hormones, tyrosine metabolism as well as monooxygenase and dioxygenase activities. Among other functions, vitamin C also helps to promote absorption of soluble non-haem iron, to reduce sensitivity to histamine and to protect food and plasma folates from oxidation (FAO/WHO, 2001).

This vitamin is synthesised in all green plants, in the liver of some mammals (including cattle), in the kidney of birds and reptiles, but not in invertebrates. However, other species like humans or non-human primates are unable to produce it due to a lack of the last enzyme of the synthetic pathway (FAO/WHO, 2001). Consequently, ascorbic acid is not considered as an essential nutrient in dairy cows as in many other species. However, in humans, vitamin C must be supplied by the diet to avoid deficiencies and their consequences, including scurvy, characterised by gingival changes, pain in the extremities, haemorrhagic events, ulcerations and death. The RDA is fixed at 90 mg/day for men and 75 mg/day for women (Table 10.1; Dietary Reference Intakes, 2000). Ths can be easily covered by consumption of fruit (mostly citrus and tomatoes) and vegetables (green leafy ones). Bovine milk could be a complementary source considering that it contains between 17 and 23 mg/L of the vitamin (Graham, 1973; Hidiroglou *et al*., 1995; Weiss, 2001; Weiss *et al*., 2004).

10.2.3 Differences in milk vitamin content between bovine and other dairy species

Dairy cows, goats and ewes produce 87% of the milk produced worldwide, but other dairy species can be of significance in some geographical areas, for example yak in the Himalayas or camelids in arid and semi-arid regions (Medhammar *et al*., 2012). Nevertheless, bovine milk is by far the one for which composition is most studied, including its vitamin content. Data are less complete for other dairy species, whether they are ruminants (ovines, caprines, buffalos or camels) or not (mare, donkey). Consequently, it is difficult to evaluate the variability of vitamin concentrations within and among species. To our knowledge, a direct comparison of the vitamin composition between dairy species with comparable physiological stages, parities and diets has never been reported, although data available in literature reviews (Hartman & Dryden, 1965; Park & Guo, 2006; Park *et al*., 2007; Raynal-Ljutovac *et al*., 2008; Medhammar *et al*., 2012) allow some comparisons.

In Table 10.2, data from the USDA database and from several references for breast milk and for milk from three

Table 10.2. Vitamin concentrations (µg/L) in milk of ruminants in comparison to human milk.

	Human		Cow		Goat		Ewe	
	Min.	**Max.**	**Min.**	**Max.**	**Min.**	**Max.**	**Min.**	**Max.**
A	390	600	295	520	400	622	438	830
D	0.02	0.40	0.30	10.00	0.57	1.10	1.80	1.80
E	800	3960	700	1100	300	700	1100	1100
K	2.5	12.7	7.5	37.6	3	3	—	—
B_1	140	200	300	450	400	680	650	800
B_2	200	360	1600	1750	1300	2100	3200	3820
B_3	1470	2200	800	955	1870	3100	4100	4270
B_5	2000	2500	3200	3500	3100	4100	3640	4500
B_6	100	110	390	600	70	600	600	800
B_8	4	50	20	60	10	39	9.3	93
B_9	7	55	19	53	2.4	30	2.4	70
B_{12}	0.1	0.5	3.5	4.0	0.6	1.0	6.0	7.1
C	40 000	50 000	7500	10 000	10 000	15 000	41 600	50 000

Sources: data from Hartman & Dryden (1965), Fournier *et al*. (1987), Elder *et al*. (2006), Park & Guo (2006), Kamao *et al*. (2007), Pandya & Ghodke (2007), Park *et al*. (2007), Raynal-Ljutovac *et al*. (2008) and United States Department of Agriculture (2012).

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ruminant species (bovine, caprine and ovine) are compared. This table illustrates that some data are lacking (even in national references tables), especially for vitamin K, and when available were sometimes obtained from a small set of samples. Several points must be emphasised about these comparisons. First, vitamin K is by far the one for which knowledge is the weakest and no comparison can really be performed, unless between breast milk and cow milk. Indeed, data analysis led to the conclusion that cow milk is richer in vitamin K than breast milk (Fournier *et al*., 1987). Data from milk of small ruminants are lacking in the literature and the USDA database only provides a value close to the lowest concentration observed in humans for goat milk (Table 10.2). Secondly, for other vitamins, concentrations are in the same range whatever the species: one to several thousand micrograms per litre for vitamins C, B_5 or B_3, several hundred for vitamins A, E or B_1, generally a few tens of micrograms per litre for vitamin B_9, and less than 10 µg/L for vitamin D and B_{12}. A more detailed comparison between species for a given vitamin suggests some differences. The milk of small ruminants (ewes and goats) is richer in vitamin A than cow milk, as a consequence of a more efficient conversion of carotenes by the tissues as demonstrated in sheep (Yang & Tume, 1993). Thus, milk fat is richer in carotenoids in bovines than in ewes or goats, leading to the yellow colour of dairy products (especially noticeable in cheeses or butter) from cows whereas those from goats and ewes are whiter. Ewe milk seems richer in vitamins than milk of other ruminants (especially cow) or human. Goat milk, in turn, is poorer in vitamin B_9 than other milks (Park *et al*., 2007; Raynal-Ljutovac *et al*., 2008). This is the reason why infants fed a diet rich in goat milk develop 'goat milk anaemia', characterised by very low levels of plasma folates (and also low levels of plasma vitamin B_{12}) that induce a megaloblastic anaemia. Consequently, the consumption of a diet based solely on goat milk by children unless supplemented with folate and vitamin B_{12} should be avoided (Ziegler *et al*., 2005; Park *et al*., 2007). Goat milk also seems poorer in vitamins D and E than others (Park & Guo, 2006; Raynal-Ljutovac *et al*., 2008). Compared to breast milk, the milk of ruminants is generally richer in vitamins D and B (especially B_1, B_2, B_5, B_6, B_{12}) but seems poorer in vitamins E and C (Table 10.2).

A review comparing milk composition among numerous dairy species was published recently (Medhammar *et al*., 2012). The authors performed a very interesting and exhaustive work using data from buffalo, yak, mithun, mare, donkey, dromedary or bactrian camels, llama, reindeer and moose milks obtained from the literature. However, concerning milk vitamins, data were lacking for yak, mithun, reindeer, llama and moose whereas partial data were available for other species. The authors compared these concentrations to those reported in cow milk by the United States Department of Agriculture (2009). One of the main observations is that dromedary milk is especially rich in vitamin C (67 000 µg/L) but also in vitamin A (970 µg/L) which is of special interest for human nutrition in areas where vegetables and fruits (the main sources of vitamin C and carotenoids in the human diet) are not easily available. By contrast, buffalo milk is reported to be 10-fold richer in vitamin B_6, twofold richer in vitamins B_3 and E but markedly poorer in vitamins B_2, B_6 and B_9 than cow milk (ranking to the level of goat milk for the latter vitamin). Concerning milk produced by mares and donkeys, reported concentrations for vitamins B_1, B_2 and B_3 were lower than for cow milk. However, mare milk seems as rich in vitamin C as ewe milk, i.e. richer than that of cow or buffalo milks but poorer than camelid milk (Medhammar *et al*., 2012).

10.3 ANIMAL AND NUTRITIONAL FACTORS MODULATING VITAMIN CONTENT IN BOVINE MILK

Most studies on the supplementation of the diet of dairy cows with vitamins were conducted to test the effects on animal performance, including production and metabolism efficiency, reproduction and immunity. However, the factors responsible for differences in milk concentrations of vitamins were relatively poorly investigated, for most of them. Retinol was by far the vitamin for which factors affecting its milk concentration were the most studied. The ranges of β-carotene and retinol concentrations in milk vary from 1 to 17 and from 1 to 12 µg/g fat, respectively, according to the literature reviewed by Nozière *et al*. (2006). The general trend is that retinol concentrations are less variable than those of β-carotene, in agreement with the complex physiological processes of regulation for the former (Nozière *et al*., 2006). Changes in concentrations of the fat-soluble vitamins A, E and β-carotene in milk are often directly explained by dietary intakes of the vitamins and/or indirectly by differences in milk fat content. For the other vitamins, only a few studies are available and understanding of factors affecting their milk concentrations is still incomplete.

10.3.1 Effects of feeding practices on vitamin concentrations in milk

Differences in milk concentrations of the fat-soluble vitamins A, E and β-carotene are mainly related to the amounts consumed by the cows. Indeed, under experimental conditions, a relationship was observed between β-carotene and vitamin E dietary intakes and their concentrations in plasma and/or secretion in milk fat (Calderón *et al*., 2007).

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These authors observed a linear dose–response between increasing dietary intakes and plasma concentrations of β-carotene and vitamin E, but in milk fat the linear increase was only observed for vitamin E not β-carotene. For the latter, milk concentrations increased until a plateau around 5 μg/g fat, suggesting saturation in the transfer from plasma to milk. These experimental results are in agreement with the literature summarised by Nozière *et al*. (2006) showing a linear relationship between β-carotene intake and concentration in milk (1.5–5.5 μg/g fat) when the dietary β-carotene content was low [0–60 mg/kg dry matter (DM)]. Results summarised by Nozière *et al*. (2006) also suggest a relationship between β-carotene dietary intake and retinol concentration in milk which, however, was not observed under the experimental conditions used by Calderón *et al*. (2007).

Changes in dietary intake of carotenoids mainly explain the differences in concentrations in milks from cows fed different types of forages or different forage-to-concentrate ratio (Nozière *et al*., 2006). Fresh forage is by far the richest source of carotenoids (1200 μg/g DM) and vitamin E (250 μg/g DM) among forages commonly used in ruminant diets. However, carotenoid content varied according to the botanical composition of the pastures; pastures rich in dicotyledons are poorer in carotenoids than other pastures with mainly monocotyledon grasses (600 vs. 1200 μg/g DM, respectively) (Nozière *et al*., 2006; Graulet *et al*., 2012). Milks with the highest concentrations of β-carotene are usually produced during spring and summer by cows at pasture, eating fresh grass. Indeed, in milk of Holstein or Montbeliarde cows at pasture, concentrations reached 5–6 μg/g fat for β-carotene and retinol (Nozière *et al*., 2006) and 0.63 μg/mL for vitamin E (Martin *et al*., 2004). These values decreased (between 2.5 and 2.8 μg/g fat for β-carotene and retinol and 0.48 μg/mL for vitamin E) when the diet was based on grass silage, hay or maize silage that are poorer in β-carotene and vitamin E (Martin *et al*., 2004; Nozière *et al*., 2006). Indeed, maize silage concentration of carotenoids varies between 20 and 70 μg/g (B. Graulet, unpublished data). The concentrate feeds are also very low in carotenoids because their ingredients are usually poor sources but also because processing, such as heating for granulation for example, destroys these compounds (Nozière *et al*., 2006). Consequently, when the proportion of concentrate in the diet is higher, such as during winter or in intensive systems, carotenoid intake is usually lower, as well as milk carotenoid concentration, than during spring and summer or in more extensive conditions (Martin *et al*., 2004; Hulshof *et al*., 2006; Agabriel *et al*., 2007). Milk concentrations of vitamins A and E are expected to follow the pattern of carotenoids because retinol is synthesised from β-carotene whereas changes in the concentration of α-tocopherol in forages and milk follow those of carotenoids as observed under experimentally controlled conditions comparing milk composition from cows receiving different diets (Martin *et al*., 2004). However, under commercial farm practices, these effects of diet composition on milk vitamin concentrations are often masked by the use of vitamin supplements (Hulshof *et al*., 2006; Agabriel *et al*., 2007). In conclusion, it seems that diet composition and use of vitamin supplements among livestock production systems that led to different micronutrient intakes explain most of the seasonal differences in milk concentrations of carotenoids and vitamins A and E.

Information on factors affecting the transfer of the other vitamins to milk is very limited. McDermott *et al*. (1985) did not observe an increase in milk vitamin D activity after dietary supplementation of cows in comparison with control cows. Until a few years ago, only a limited amount of research on B vitamin requirements of dairy cows was conducted because supply from both dietary intakes and bacterial rumen synthesis was considered sufficient to cover the cow's estimated requirements. However, from 1990 to 2005, cow milk and milk component yields, and consequently nutrient demand, increased by about 33% while DM intake increased by only 15% (Weiss & Ferreira, 2006). As presented in section 10.2.2.1, B vitamins act as cofactors in most major metabolic pathways. Consequently, it is very likely that the demand for these vitamins increased with production performance. Nevertheless, despite renewed interest in dairy cow requirements for B vitamins, information on the effect of B vitamin supply to dairy cows on transfer to milk is very limited. Milk concentrations of B vitamins seem generally poorly related quantitatively to their intakes from feed ingredients (Haug *et al*., 2007) probably as the result of degradation and synthesis of these vitamins in the rumen. Indeed, recent works showed that duodenal flow and ruminal synthesis of B vitamins are modified by the composition of the diet but are poorly related to vitamin intake (Schwab *et al*., 2006; Seck *et al*., 2010a, b, 2011). Unfortunately, the effects on milk vitamin concentrations are not available in these papers. Dietary supplements of synthetic B vitamins are extensively destroyed in the rumen (Santschi *et al*., 2005). Nevertheless, when dietary supplements of folic acid and vitamin B_{12} at levels similar to those studied by Santschi *et al*. (2005) were fed to lactating cows, they increased secretion of these two vitamins in milk (Graulet *et al*., 2007), suggesting that a fraction of the dose bypassed degradation in the rumen. However, in these two latter experiments, high doses of supplements were given. Similarly, supplementations with pantothenic acid (vitamin B_5) protected from bacterial degradation in the rumen increased its plasma concentration in dairy cows (Ragaller *et al*., 2010, 2011). Nevertheless, unprotected vitamin B_5 had only marginal

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effects, especially at the lower doses, and transfer to milk was not affected suggesting that the vitamin was mostly destroyed by ruminal microflora. Interestingly, recent results observed on commercial farms have indicated that milk concentrations of vitamin B_9 and vitamin B_{12} varied according to the feeding system. Indeed, the vitamin B_{12} content was highest (up to +32%) in the milk at farms where cows were fed maize-rich diets whereas milk produced with diets rich in hay in winter or pasture during the grazing period were richer in vitamin B_9 (Chassaing *et al*., 2011). These results could be explained by different dietary intakes of these vitamins according to diet composition or more likely by the effects of diet composition on bacterial populations and/or fermentations altering bacterial synthesis of these vitamins. Recent results confirm that the milk concentration of B-complex vitamins can be modified. However, for practical application, increasing the milk concentration of B vitamins would require either a better understanding of dietary factors affecting synthesis of these vitamins in the rumen or use of rumen-protected B vitamins.

10.3.2 Non-dietary factors affecting milk concentrations of vitamins

Knowledge of non-dietary factors affecting milk concentrations of vitamins is greater for vitamin A than for the other vitamins. The effects of breed, stage of lactation, health status of the udder, milk and fat yields, and genetic traits on milk concentrations of vitamin A have been reviewed by Nozière *et al*. (2006). Among dairy breeds, large differences were reported for β-carotene concentrations according to the Jersey-to-Friesian gene ratio, especially when fed carotenoid-rich diets. The general trend is that Jersey and especially Guernsey cows produce milk with carotene concentrations twofold to threefold higher than in milk of Holstein-Friesian, Brown Swiss, Ayrshire or Shorthorn cows. Plasma concentrations of carotene followed the same pattern, suggesting that the breed effect was not a consequence of the efficiency of the mammary gland in transferring β-carotene from plasma to milk but was rather due to an effect on carotene absorption and/or metabolism. On the other hand, retinol concentrations are higher in milk of Holstein-Friesian than in Guernsey cows, whereas other comparisons among breeds such as Holstein, Montbeliarde and Tarentaise, Jersey and Holstein, Holstein-Friesian, Brown-Swiss and Jersey did not show significant differences. To summarise, differences among breeds for vitamin A content in milk are due to changes in carotene rather than retinol concentrations.

Heritability of the milk carotenoid or retinol concentrations has been reported according to sire. Thus, genetic selection of bulls based on milk concentrations of these components could be a way to improve the nutritional composition of the milk.

The physiological sources of the variability between animals could be direct, via genetically determined transfer of the compounds from the digestive tract to the milk (at the level of intestinal absorption, cellular conversion of carotenes, plasma uptake by the mammary gland, etc.) or indirect, through milk and milk fat yields (by a dilution–concentration effect). For example, β-carotene concentration in milk fat is lower for dairy cows in their second lactation as compared to primiparous cows but it increased thereafter with lactation number. In these conditions, it was shown to be inversely related to milk yield and positively related to milk fat content. These relationships could possibly explain changes in milk concentrations of β-carotene throughout lactation, although there are confounding effects between stage of lactation and dietary intakes and diet composition. The information on changes in vitamin secretion by the mammary gland at the onset of the lactation is limited. For carotenoids and retinol, concentrations are much higher in colostrum (during the first 3–4 days of lactation) than in milk (Nozière *et al*., 2006) whereas milk pantothenic acid is low the day after calving but quickly increases to reach maximal values between days 4 and 14 of lactation (Ragaller *et al*., 2011). Data from 12 Holstein dairy cows showed that milk concentrations of vitamin B_{12} decreased between weeks 4 and 8 after calving (from 2200 to 1400 pg/mL), whereas milk folates increased (from 37 to 48 ng/mL) (B. Graulet & C.L. Girard, unpublished data). More studies are needed to identify the factors responsible for changes in milk concentrations of vitamins throughout lactation.

10.4 VITAMIN CONTENT IN CHEESES

Vitamin concentrations in cheeses are highly variable and very different from those in milk. The concentrations of fat-soluble vitamins in cheeses depend mostly on their fat content. Most fat-soluble vitamins present in milk pass into cheese regardless of the type of cheesemaking process. The proportion of each vitamin initially present in milk but lost during the cheesemaking process ranges from 15 to 35% for vitamin A and approximately 65% for vitamin E (Renner, 1993; Lucas *et al*., 2006). The losses may result from their oxidative degradation by oxygen and light (Erdman *et al*., 1988) and their high sensitivity to photoisomerisation following light exposure of milk and cheese during cheese manufacturing and storage (Zahar *et al*., 1987). The partial loss of vitamin A into the whey during the cheesemaking process could also be linked to the ability of some whey proteins, i.e. β-lactoglobulin or α-lactalbulin, to form water-soluble complexes with retinol (Puyol *et al*., 1991). For vitamins A and E, when reported for different varieties of cheeses with a similar fat

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content, the variability observed is mostly explained by the milk concentrations of these vitamins and factors affecting them have been described previously. Lucas *et al*. (2006) observed a linear correlation between the concentrations of vitamins A and E in cheese and milk fat; the r^2 values were 0.54 and 0.81 for vitamins A and E, respectively.

In contrast, a major proportion of the water-soluble vitamins initially present in milk is lost into whey, with only 10–20% of thiamin, nicotinic acid, folic acid and ascorbic acid present in milk recovered in cheese. These values are slightly higher for riboflavin and biotin (20–30%), pyridoxine and pantothenic acid (25–45%) and cobalamin (30–60%) (Reif *et al*., 1976; Renner, 1993). Cheese concentrations of water-soluble vitamins depend mostly on cheese moisture content. However, even after correction for moisture content, a high variability among cheese varieties is still reported (Shahani *et al*., 1962) and it is generally not influenced by milk composition (Lucas *et al*., 2006). Depending on cheese varieties, bacteria and moulds have different abilities to use and to synthesise some water-soluble vitamins, particularly B vitamins. B-vitamin concentrations in cheese can also vary according to the type of starter cultures used and generally increase with time of storage (Scott & Bishop, 1988). It has been shown by isolating individual microorganisms from cheeses that some were able to synthesise niacin, folic acid, biotin and pantothenic acid. In hard cheese varieties like Emmental, synthesis of vitamin B_{12} by propionic acid bacteria has been well described (Hugenholtz *et al*., 2002). Moreover, the moulds from the outer layers and the core of some cheese varieties are also responsible for an important synthesis of B vitamins. Consequently, small-size cheeses like some goat milk cheeses or Camembert cheese that have a proportionally high outer layer and which are consumed entirely without removing the rind, are generally rich in B vitamins (particularly vitamins B_2, B_6, B_9 and B_{12}) (Lucas *et al*., 2006; Raynal-Ljutovac *et al*., 2008). Blue cheeses are also rich in B vitamins. On the other hand, most of ascorbic acid is degraded during cheese ripening, so ripened cheese varieties are devoid of vitamin C.

10.5 CONCLUSIONS

The consequences of suboptimal intakes of vitamins on human health have been described. It would be interesting to improve the nutritional quality of foods, especially milk and dairy products, by optimising their vitamin content, not only for fat-soluble vitamins but also folates, riboflavin and cobalamins (Smilowitz *et al*., 2005). Increasing the nutritional density of milk and dairy products would make them a very attractive source of vitamins (Drewnowski, 2011), especially for the very young and the very old who have limited food intake and often reduced absorption efficiency.

In this chapter, the availability of vitamins in milk and their nutritional benefits have been presented, as well as the importance of milk in meeting the RDA for each vitamin. There are huge differences among vitamins. In human diets, some of the vitamins are mostly supplied by animal products (vitamins A, D, B_2, B_5 and B_{12}), whereas fruits and legumes are major sources of others. Factors affecting retinol concentrations in milk are particularly well known, especially in bovines, whereas for vitamins D and K, for example, information is too scarce to understand how their secretion in milk is regulated. The situation is similar for B vitamins for which supplementary information is needed to identify the factors regulating (i) their synthesis and degradation in the rumen and (ii) their transfer to milk. Such is also the case for other dairy species.

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11
Milk Minor Constituents, Enzymes, Hormones, Growth Factors, and Organic Acids

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11.1 INTRODUCTION

Milk and derived products contain several essential nutrients and protective agents, such as enzymes and growth factors (Fox & Kelly, 2006a). However, lately a decrease in milk consumption in Western societies has been observed, mainly due to negative effects on health that have been claimed regarding its intake (Haug *et al*., 2007). The high content of saturated fatty acids in milk has been pointed out as being responsible for negative effects contributing to heart diseases, weight gain, and obesity (Insel *et al*., 2004). Nevertheless, this issue is controversial since there are many milk components that promote health benefits, including oleic acid, conjugated linoleic acid (CLA), omega-3 fatty acids, proteins, vitamins, minerals and bioactive compounds, and various milk proteins and their peptides have been suggested to possess anticancer activity (Rodrigues & Teixeira, 2009; Rodrigues *et al*., 2009; Duarte *et al*., 2011). Regarding milk fat, an increase in mean gastric emptying time has been observed when comparing the consumption of whole with half-skim milk, so that whole milk promotes an increase in gastrointestinal transit time. Therefore, the consumption of whole milk may be valuable for regulating appetite and it has not been proven that moderate consumption of milk fat is related to an increased development of certain diseases. The relationship between milk or milk product consumption and possible negative health effects is still not fully explored. For example, the interaction between carbohydrates and protein in milk exposed to heat may result in products whose effects have not been accurately assessed. The association between food and health is well established (Kussmann & Fay, 2008) and some studies have shown that variable risk factors seem to be of greater significance for health than previously anticipated (Yusuf *et al*., 2004). Prevention of disease may in the future be just as important as treatment of diseases (Torres *et al*., 2010). Indeed, currently many consumers are extremely conscious of the health properties of food, and the market for healthy food and food with special health benefits is increasing (Haug *et al*., 2007).

Milk is a complex matrix made up of components, which per se may have negative or positive health effects, respectively. The concentration in milk of several nutrients, including the minor constituents, can be manipulated through feeding regimes and it can be increased or diminished to produce a healthier product. The development of functional and healthy foods has become one of the most exciting fields of research in recent years (Michaelidou & Steijns, 2006; Pouliot & Gauthier, 2006; Kaput, 2008; Michaelidou, 2008). The relative ease with which milk can be converted into a wide variety of products makes it an extremely useful base material. In some cases, milk undergoes relatively limited processing,

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consisting of heat treatment to increase the product microbial shelf-life and homogenization to increase the physical shelf-life through retarding fat separation (Huppertz & Kelly, 2009). Other well-known processes involve the acid-induced coagulation of milk to produce yogurt, or the enzymatic coagulation of milk to manufacture cheese. In addition, milk may be spray-dried or used as a base from which constituents, such as proteins, fats or minor constituents, are isolated. As a result of the widespread applications and use of milk and milk products in human nutrition they have been the subject of intensive research in the last century.

This chapter focuses on the description of the minor constituents, enzymes, hormones, growth factors and organic acids in milk, as well as their applications and technological challenges. Finally, future perspectives and concerns related to these constituents will be discussed.

11.2 MILK MINOR CONSTITUENTS

Milk is often described as a colloidal suspension, containing emulsified globules of fat, a heterogeneous family of major and minor proteins, the carbohydrate lactose, minerals, vitamins, enzymes (Huppertz & Kelly, 2009) and many other minor components that hold important physiological and/or technological roles such as immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes, and other bioactive peptides (Haug *et al*., 2007). The lipids in milk are emulsified in globules coated with membranes. The proteins are in colloidal dispersions as micelles. The casein micelles occur as colloidal complexes of protein and salts, primarily calcium (Keenan & Patton, 1995). Lactose and most minerals are in solution. Milk composition has a dynamic nature that varies with several factors (Ontsouka *et al*., 2003; Huppertz & Kelly, 2009):

1. Genetics (e.g. species, breed and individual).
2. Stage of lactation.
3. Health status of the individual animal.
4. Environmental factors (e.g. feed, climate or method of milking).

Specific milk proteins are involved in the early development of the immune response, whereas others take part in non-immunological defense (e.g. lactoferrin) (Rodrigues *et al*., 2009). In addition to the major constituents, milk also contains a number of organic and inorganic compounds in small or trace amounts (e.g. peptides, amino acids, antioxidants, salts, nucleotides and vitamins), some of which affect both the processing and nutritional properties of milk. Table 11.1 summarizes the amounts of some of the most important minor milk constituents in milk from several species.

11.2.1 Salts and minerals

Milk salts are essentially phosphates, citrates, chlorides, sulfates, carbonates, and biocarbonates of sodium, potassium, calcium and magnesium. Since milk contains organic and inorganic salts, the level of salts is by no means equivalent to the ash content (Huppertz & Kelly, 2009). The milk salt composition is also influenced by a number of factors, including species, breed, stage of lactation, health status, climate, and feed.

Although salts comprise less than 1% of the milk, they influence its rate of coagulation and other functional properties. Calcium, magnesium, phosphorus, and citrate are distributed between the soluble and colloidal phases. Their equilibrium is altered by heating, cooling and by a change in pH. The solubility of calcium phosphate is strongly temperature dependent and, unlike for most other compounds, decreases with increasing temperature (Pouliot *et al*., 1989). In their studies, these authors found that the levels of calcium and phosphate in the milk serum decrease progressively with temperatures increasing in the range 4–90°C. Heat-induced decreases in levels of serum magnesium and citrate were also observed but to a smaller extent. Levels of sodium and potassium in milk serum were not affected by heat treatment. Severe heat treatments (>90°C) may result in irreversible changes in the mineral balance in milk (Holt, 1995).

In addition to the major salts, milk also contains trace elements. Some elements come into the milk from feeds, but milking utensils and equipment are important sources of such elements as copper, iron, nickel, and zinc. Mineral and vitamin contents of goat and sheep milk are mostly higher than in cow milk (Park *et al*., 2007).

11.2.2 Vitamins

The fat-soluble vitamins A, D, E and K in milk are associated with the milk fat fraction, while the water-soluble B-complex vitamins and vitamin C are associated with the water phase. Vitamins are unstable and processing can therefore reduce the effective vitamin content in milk. During processing, the fat-soluble vitamins are retained by the cream, while the water-soluble vitamins remain in skim milk or whey (Michaelidou & Steijns, 2006).

Several researchers have suggested that slight deficiencies in B vitamins may constitute risk factors for vascular and neurological diseases and cancers (Brachet *et al*., 2004). A combined deficiency of folates and vitamin B_{12} has been associated with the development of dementia and Alzheimer's disease among the eldery (Seshadri *et al*., 2002). Furthermore, folate, B_6 and B_{12} influence homocysteine metabolism. As elevated levels of plasma homocysteine constitute a risk factor for developing cardiovascular

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Table 11.1. Contents of some minor constituents in milk from several species.

	Cow	Human	Goat	Sheep	Buffalo	Mare	Camel
Minerals and trace elements (mg/kg)							
Calcium (Ca)	1190–1220	330	1340–1500	1930	1830	485–1355	760–1965
Phosphorus (P)	930–1190	430	960–1210	1580	820	216–1205	490–1480
Potassium (K)	1520–1520	550	1700–1810	1360	1070	303–790	600–2110
Sodium (Na)	490–580	150	410	440	440	75–237	360–902
Magnesium (Mg)	120–130	40	93–160	180	180	29–118	40–209
Chlorine (Cl)	1000–1030	600	1500		580		
Sulfur (S)	320		29		157–314		
Zinc (Zn)	3.8–5.3	3.8	2.7–5.6	5.7	3.2–7.3	2.2–3.6	2.8–4.4
Iron (Fe)	0.5–0.8	2.0	0.7–4.0	0.8	0.4–13	0.5–1.1	0.–3.7
Copper (Cu)	0.2–0.6	0.6	0.5–1.0	0.4	0.07–2.6	0.2–0.7	0.11–1.5
Manganese (Mn)	0.2–0.3	0.7	0.3–1.0	0.07	0.38–0.66	0.05	0.2–1.9
Aluminum (Al)	0.6		1.12	0.5–1.8			
Cadmium (Cd)	0.004		0.005	0.03–0.06			
Cobalt (Co)	0.0008			0.004–0.09	0.7–1.6		
Chromium (Cr)	0.02			0.04–0.4			
Nickel (Ni)	0.02		0.288	0.01–0.4			
Barium (Ba)	0.2			1.7			
Lead (Pb)	0.03			0.006			
Selenium (Se)	0.0096–0.02	0.0152	0.013–0.247				
Fluorine (F)	0.1				0.4–18.5		
Iodine (I)	0.08/0.21	0.07	0.22		8.6–19.4		
Molybdenum (Mo)	0.06		0.116				
Vitamins (mg/kg)							
Retinol, vitamin A	0.38–0.52	0.57	0.55	0.84	0.102	0.34	
Thiamin, vitamin B_1	0.28–0.90	0.14–0.17	0.68	0.8	0.5		0.33–0.6
Riboflavin, vitamin B_2	1.2–2.0	0.2–0.36	2.1	3.56	1.0		0.42–0.8
Pyridoxine, vitamin B_6	0.42–0.63	0.11	0.46	0.8	3.8		0.52
Cobalamin, vitamin B_{12}	0.002–0.007	0.0003–0.0005	0.0007	0.007	3.4		0.002
Vitamin D	0.0003–0.0005	0.0004	0.0006	0.0018		0.0032	
Tocopherol, vitamin E	0.31–0.9			1.1	0.334	1.128	
Ascorbic acid	3–23	35–50	12.9	41.6	23–30	17.2	24–52
Folic acid	0.01–0.1	0.055	0.01	0.05	0.1		0.004
Niacin	0.5–0.84	1.47–1.7	2.7	4.16			4–6
Pantothenic acid	2.6–4.9	1.84–2.23	3.1	4.08	1.5		0.88
Biotin	0.02	0.004	0.015		26.8		
Immune components							
Immunoglobulins (%)	10.1–11.7	15.1–19.7				18.7–20.9	

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IgA (milk: μg/mL)	140	1000	30–80				
IgA (colostrum: mg/mL)	3.9	17.35	0.9–2.4				
IgM (milk: μg/mL)	50	100	10–40				
IgM (colostrum: mg/mL)	4.2	1.59	1.6–5.2				
IgG (milk: μg/mL)	590	40	100–400	350–500	450–600		1700
IgG (colostrum: mg/mL)	47.6	0.43	50–60				
Lactoferrin (μg/mL)	20–200	<2000	20–200	20–200	20–200	20–200	150–250
Polyamines (μmol/L)							
Putrescine	1	0.4	5.12–6	0.4			
Spermidine	1–4.7	2.7	26–39.67	2.05			
Spermine	1–4	1	3.18–3.8	2.39			
Nucleosides (μmol/L)							
Cytosine	2.4–5.8	4.3–7.8	8.8	6.7			
Uridine	14.7–73.1	0.5–6.9	17.9–76.3	67.8			
Inosine	1–6.5		12.8–60.6	41.2			
Guanosine	0.8	0.2–1	2.3–2.9	2.1			
Adenosine	1.4	3–5.3	2.4–3.4	8.8			
Nucleotides (μmol/L)							
Cytidyl-5′-monophosphate	2.9–26.6	18.3–66	72.5	48.6			
Uridyl-5′-monophosphate	Traces	6.4–9.3	227.2	110.7			
Guanosyl-5′-monophosphate	1.8	1–1.5	Traces	Traces			
Adenosyl-5′-monophosphate	Traces	1.9–15.1	85.6	54.1			
Essential amino acids							
Alanine (g/kg)	1.08	3.42	0.89	2.17		0.59	
Arginine (g/kg)	1.15–1.98	3.09	0.75	1.2–1.84		0.95	3.92
Cysteine (g/kg)	0.31	1.73	0.23	0.44	0.59	0.18	
Glycine (g/kg)	0.61	1.89	0.47	0.98		0.25	
Histidine (g/kg)	1.68–8.10	1.97–2.17	0.67–2.23	0.88–1.41		0.35	1.95–2.15
Isoleucine (g/kg)	1.58–3.38	2.33–4.53	1.24–2.15	2–2.66	5.71	0.62	2.94–5.71
Leucine (g/kg)	3.33–5.88	2.71–8.85	2.48–2.68	3.22–4.89	9.79	1.47	2.71–8.55
Lysine (g/kg)	2.90–5.12	5.36–6.09	2.06–5.48	2.6–4.51	7.50	1.16	3.75–4.26
Methionine (g/kg)	0.88–1.56	1.36–1.46	1.43	0.84–1.57	0.93	0.35	2.42–2.60
Phenylalanine (g/kg)	1.68–2.84	2.58–3.15	1.21–1.99	1.6–2.6	4.71	0.68	3.15–3.84
Threonine (g/kg)	1.41–2.68	3.56–3.75	1.26–3.31	1.48–2.22	3.57	0.62	2.39–2.51
Tryptophan (g/kg)	0.84	5.22	5.11	0.46			12.53
Tyrosine (g/kg)	1.58	3.93	0.98	2.55	3.86	0.72	
Valine (g/kg)	1.75–4.48	4.35	1.57	2.2–3.10	6.74	0.75	3.39
Branched-chain amino acids (g/kg)	7.02–13.64	1.78	5.29	7.44–10.77		2.81	
Taurine (μmol/dL)	1.0	30	56	14			

Sources: based on data from Park & Haenlein (2006), Dimitrov *et al*. (2007), Michaelidou (2008), Belewu & Adewole (2009), Csapó *et al*. (2009) and Haenlein & Anke (2011).

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disease, an increase in folate intake would be beneficial (Graham & O'Callaghan, 2000). Apart from the prevention of cardiovascular diseases, folates possess a protective role against child birth defects (Forssen *et al*., 2000; Molloy, 2002). Also, there is growing evidence that low folate status is linked to an increased cancer risk, particularly colon cancer (Rampersaud *et al*., 2002). Based on the evidence that B vitamins are beneficial for human health, they have been included in the list of nutraceuticals (Hugenholtz *et al*., 2002).

At an industrial scale, lactic acid bacteria (LAB) are used to increase the production levels of B vitamins in dairy products. Recent developments in LAB metabolic engineering include the re-routing of complex biosynthetic pathways leading to the production of metabolites with a health benefit for the consumer, as is the case for the B vitamins. These advances could lead to novel functional foods with great potential for the application of LAB (Kleerebezem & Hugenholtz, 2003). Novel dairy foods, enriched through fermentation using multivitamin-producing organisms with mutations in methylene tetrahydrofolate reductase, could compensate the B vitamin deficiencies known worldwide and could specifically be used in dietary foods for specific consumer groups (Sybesma *et al*., 2004).

Using fermentation processes for the natural enrichment of foods presents key advantages over the enrichment of food through the addition of chemically synthesized vitamins. It enables the use of naturally occurring molecules in physiological doses and in an environment most suited for optimum biological activity (Michaelidou & Steijns, 2006). This is of extreme importance for dairy products, since some bioactive molecules do not work alone, requiring their specific binding proteins or other milk proteins for biological activity. Additionally, it is important to notice that the use of these fortified fermented foods generally is not limited by legislation. However, as in all cases of food fortification, special care should be given to possible adverse effects related to the excess intake of vitamins.

11.2.3 Immune components

Milk plays an important role in mammalian host defense (Stelwagen *et al*., 2009). Present in colostrum and milk of all lactating species, immunoglobulins provide immunological protection of the offspring against microbial pathogens and toxins. Depending on the species, one can find different types of immunoglobulins and diffferent concentrations. In colostrum, the concentration of immunoglobulins is particularly high, with IgG being the major immunoglobulin class present in ruminant milk, in contrast to human milk in which IgA is the major immunoglobulin. Immunoglobulins are transported into mammary secretions via specialized receptors. In addition to immunoglobulins, both colostrum and milk contain viable cells, including neutrophils and macrophages, which secrete a range of immune-related components into milk. These include cytokines and antimicrobial proteins and peptides, such as lactoferrin, defensins, and cathelicidins. Mammary epithelial cells themselves also contribute to host defense by secreting a range of innate immune effector molecules. A detailed understanding of these proteins and peptides offers great potential to add value to the dairy industry. This is demonstrated by the widespread commercial applications of lactoferrin isolated from bovine milk (Rodrigues *et al*., 2009). Furthermore, some immunoglobulins may influence milk processing, as is the case of IgM that plays an important role in the creaming of cow milk (Huppertz & Kelly, 2009). Sheep and goat milk are also important sources of minor milk proteins with immune effects, including immunoglobulins, lactoferrin, transferrin, ferritin, proteose peptone, calmodulin (calcium-binding protein), prolactin, and folate-binding protein. Non-protein nitrogen (NPN) content of goat and human milks is higher than in cow milk (Park *et al*., 2007).

11.2.4 Bioactive peptides

Because of advances in biological research tools, milk and whey proteins have been recognized to contribute to human health through latent biological activity (Rodrigues & Teixeira, 2009). These proteins are hydrolyzed by certain proteolytic enzymes releasing the so-called bioactive peptides that are capable of modulating specific physiological functions (Michaelidou, 2008).

Bioactive peptides can be obtained from precursor proteins through enzymatic hydrolysis by digestive enzymes derived from microorganisms or plants, or through fermentation of milk with proteolytic starter cultures (Korhonen & Pihlanto-Leppala, 2006). These peptides have been shown to exert various activities affecting the digestive, cardiovascular, immune, and nervous systems. Specifically, antihypertensive, antioxidative, antithrombotic, and hypocholesterolemic peptides can affect the cardiovascular system. Opioid peptides, with agonist or antagonist activity, may regulate the nervous system. Mineral-binding, anti-appetite, and antimicrobial peptides exert their action on the gastrointestinal system. Immunomodulatory and cytomodulatory peptides are of special interest for the immune system. The occurrence and biological activity of these peptides in milk and its derivatives has been extensively reviewed (Clare & Swaisgood, 2000; FitzGerald & Meisel, 2000; Meisel & FitzGerald, 2000; Kilara & Panyam, 2003; FitzGerald *et al*., 2004; Korhonen & Pihlanto-Leppala, 2006; Lopez-Fandino *et al*., 2006).

Among the bioactive peptides from milk, those with blood pressure-lowering effects are receiving special

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attention (FitzGerald *et al*., 2004). Some antihypertensive products based on milk peptides with clinically proven health benefits are currently available in the market (Lopez-Fandino *et al*., 2006). Sheep and goat milk proteins are important sources of bioactive angiotensin-converting enzyme (ACE)-inhibitory peptides and antihypertensive peptides (Park *et al*., 2007). Goat milk is being regarded as an appealing research area, as it has been less explored than bovine milk, but also because novel peptidic ACE inhibitors have been found in goat milk hydrolyzates (Geerlings *et al*., 2006). As sheep milk is usually converted to cheese, and mostly to traditional cheeses, ovine cheese varieties can be regarded as a valuable source for ACE-inhibitory peptides. The presence of hypotensive peptides, naturally formed in cheese, has been found to depend on a balance between their formation and their degradation (Ryhanen *et al*., 2001). Further research has to be conducted to evaluate the long-term physiological effects of consuming such peptides.

Another group of milk bioactive peptides that has aroused the interest of the scientific community is the caseino-phosphopeptides (CPPs), since they have been suggested to enhance vitamin D-independent bone calcification in rachitic infants (Mellander, 1950). Its mechanism of action seems to be related to the presence, in their amino acid sequence, of a cluster of three phosphoserine residues followed by two glutamic acid residues. This sequence produces, at the intestinal pH, a negative core responsible for mineral binding (Ca, Zn, Mg) and for resistance of these peptides to proteolytic gastrointestinal enzymes. These two structural features support the hypothesis that CPPs could increase passive diffusion and utilization of calcium *in vivo* by increasing calcium solubility at physiological pH of the distal small intestine. However, results from *in vivo* studies are still controversial, as there are many factors that could affect calcium availability, such as the various dietary compounds present at the same time in the intestinal lumen (FitzGerald, 1998; Vegarud *et al*., 2000; Erba *et al*., 2001).

Components that are able to transmit biochemical messages have attracted particular scientific attention as potential bioactive ingredients in a range of biomedical and functional foods, since they have been shown to be potent growth stimulants and mediators for a range of mammalian cells, both *in vitro* and *in vivo* (Smithers, 2004). Among them, non-peptide trophic factors play an important role in maintaining gastrointestinal mucosal mass and modulating the immune system via multiple mechanisms (Playford *et al*., 2000). These factors include glutamine, polyamines, and nucleotides.

11.2.5 Polyamines

The interest in naturally occurring polyamines in milk, such as putrescine, spermidine and spermine, has been increasing in the last decades (Michaelidou, 2008). Polyamines consist of flexible polycations that are fully charged under physiological pH conditions. They fulfill a number of roles in cellular metabolism and are essential for cell growth and proliferation (Löser, 2000; Eliassen *et al*., 2002; Gugliucci, 2004; Larqué *et al*., 2007). Besides being involved in DNA, RNA and protein synthesis, the most important function of polyamines is the mediation of the action of all known hormone and growth factors.

The polyamine requirements that cannot be met by biosynthesis have to be satisfied by exogenous polyamines consumed from the food (Jeevanandam *et al*., 1997). It has been suggested that gut maturation is sustained by dietary polyamines; therefore its supplementation in formula-fed infants may prove beneficial. Dietary polyamines may therefore decrease absorption of cows' milk allergen and reduce the risk of food allergy (Dorhout & Muskiet, 1999).

Furthermore, polyamines may be important for the fidelity of the enhanced DNA transcription and RNA translation that occurs in response to infection and during tissue repair, gut growth after surgery, and in gut barrier functions (Grimble & Grimble, 1998). Additionally, dietary polyamines might become important with aging as cell proliferation slows with age (Nishimura *et al*., 2006). Conversely, there are situations where diets low in polyamines may be beneficial such as in the treatment of some tumors (Gugliucci, 2004; Larqué *et al*., 2007). Accordingly, the effect of polyamines on human health may vary among people.

11.2.6 Nucleotides

Nucleotides, nucleosides, and nucleobases belong to the NPN fraction of milk and are known to have a specific physiological impact in early life (Michaelidou, 2008). Nucleosides and nucleobases, the preferred forms for absorption in the intestine, are suggested to be the active components of dietary and/or supplemented nucleic acid-related compounds in the gut. Schlimme *et al*. (2000) reviewed the composition and biological activity of these minor constituents in bovine milk and colostrum. Because of the properties and roles of dietary nucleotides, an increased interest in their use for infant nutrition has been registered, and supplementation with ribonucleotide salts in the manufacture of infant and follow-on formulae has been allowed by the European Commission (Gil & Rueda, 2002; Yu, 2002; Aggett *et al*., 2003; Alles *et al*., 2004).

Recently it has been found that modified nucleosides may inhibit cell proliferation and activate apoptosis. Food-derived inducers of apoptosis may be of significance as exogenous anticarcinogens in the control of malignant cell proliferation, where the intestinal tract could be the primary target site for a possible selective apoptotic stimulant against malignant cells (Schlimme *et al*., 2000).

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Furthermore, dietary nucleotides influence biosynthetic processes and modulate gene expression (Sanchez-Pozo & Gil, 2002). Functions of the system and the brain also appear to benefit from food supplementation with nucleosides and nucleotides (Yamamoto *et al*., 1997), although its effects in the gut seem to depend on the type of damage.

11.2.7 Proteose peptones

The potential exploitation of selected milk proteins as ingredients in functional food products has been the reason for an increasing interest in their fractionation (Rodrigues & Teixeira, 2009; Zuniga *et al*., 2009). Heating of skim milk (95°C, 30 min) followed by acidification promotes the denaturation of whey proteins and their co-precipitation with caseins, which are insoluble at pH 4.6 (Girardet & Linden, 1996). Despite these drastic conditions, a heterogeneous fraction called proteose peptone (PP) remains soluble (Huppertz & Kelly, 2009). The PP fraction of milk appears to consist of two groups of proteins/peptides, i.e. those that are endogenous in milk (e.g. osteopontin, proteose peptone 3), and those that result from the action of proteolytic enzymes (primarily plasmin, on caseins). The principal components of the PP fraction have been designated components 3, 5, and 8 (PP3, PP5, PP8) (Innocente *et al*., 1999).

The primary structure of PP3 includes a polypeptide backbone of 135 amino acid residues containing five phosphorylated serines, two threonine-linked *O*-glycosylations, and one *N*-glycosylation, with an apparent molecular mass of 28 kDa (Sorensen *et al*., 1997). Also, a glycoprotein with apparent molecular mass of 18 kDa is associated with component PP3, corresponding to the 54–135 fragment released by plasmin hydrolysis in milk (Sousa *et al*., 2007). PP3 is extremely hydrophobic and particularly interesting because of its functional properties, such as its emulsifying power, strong affinity for oil–water interface, strong foaming properties, and biochemical role (Rodrigues *et al*., 2003). Although not many studies have been conducted on the biological functions of PP3, some researchers demonstrated its immunostimulation (Sugahara *et al*., 2005) and prebiotic effects (Etienne *et al*., 1994), as well as its role in caries prevention (Grenby *et al*., 2001; Aimutis, 2004).

11.2.8 Branched-chain amino acids and other amino acids

Milk is rich in essential amino acids and branched-chain amino acids (Haug *et al*., 2007). These amino acids have unique roles in human metabolism; in addition to providing substrates for protein synthesis, suppressing protein catabolism and serving as substrates for gluconeogenesis, they also trigger muscle protein synthesis and promote protein synthesis (Wolfe, 2002; Layman, 2003; Etzel, 2004).

The branched-chain amino acid leucine in particular triggers muscle protein synthesis that is sensed by the insulin signaling pathway (Etzel, 2004). The stimulated insulin secretion caused by milk is suggested to be caused by milk proteins, and as shown by Nilsson *et al*. (2007) a mixture of leucine, isoleucine, valine, lysine, and threonine resulted in glycemic and insulinemic response resembling the response seen after ingestion of whey. A combination of milk with a meal with high glycemic load (rapidly digested and absorbed carbohydrates) may stimulate insulin release and reduce the postprandial blood glucose concentration (Frid *et al*., 2005). A reduction in postprandial blood glucose is favorable, and it is epidemiological evidence suggesting that milk may lower the risk of diseases related to insulin resistance syndrome (Pereira *et al*., 2002).

11.2.9 Taurine

Taurine is an essential amino acid for preterm neonates and for specific consumer groups that are at risk for taurine deficiency, such as patients requiring long-term parenteral nutrition (including premature and newborn infants), diabetic patients, and those with chronic hepatic, heart or renal failure (Lourenco & Camilo, 2002; Li *et al*., 2005). It is suggested that during parenteral nutrition, taurine supplementation (50 mg/kg body weight) may be required. Park *et al*. (2007) reported that the taurine concentration in normal cow milk is 0.6 mg/dL (1 μmol/dL), while the concentration in cow colostrum is 8 mg/dL. Human mature milk contains significantly more taurine (30 μmol/dL) than cow milk or milk from other species, such as sheep (14 μmol/dL), but is similar to goat (56 μmol/dL) (Park *et al*., 2007; Belewu & Adewole, 2009). Taurine content of milk can be increased through feeding, although there are some issues with its degradation as was suggested by Kim and Park (2003) in their patent (US Patent 6645519).

Taurine is the most abundant intracellular amino acid in humans. It may be synthesized in the body from methionine and cysteine, but in healthy individuals milk in the diet is the usual source of taurine. It is implicated in numerous biological and physiological functions, such as bile acid conjugation and prevention of cholestasis, antiarrhythmic/inotropic/chronotropic effects, central nervous system modulation, retinal development and function, endocrine/metabolic effects, and antioxidant/anti-inflammatory properties (Lourenco & Camilo, 2002). This essential amino acid has been shown to have endothelial protective effects (Fennessy *et al*., 2003), and it may function principally as a negative feedback regulator, helping to dampen immunological reactions before they cause too much damage to host tissues or to the leukocytes themselves (Park *et al*.,

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2002); it has also been shown to be analgesic (Silva *et al*., 1993; Li *et al*., 2005).

11.2.10 Glutathione

Milk is a good source of glutathione, which acts in the organism as an antioxidant. Glutathione is a tripeptide of the sulfur amino acid cysteine, plus glycine and glutamic acid. It can be oxidized to form oxidized glutathione, and in this reaction it may remove reactive oxygen species (ROS), thereby regulating the level of ROS in the cells. Glutathione participates in the regulation of insulin production in pancreatic cells, as ROS inhibit expression of the proinsulin gene. Glutathione appears to have important roles in leukocytes, as a growth factor, as an anti-apoptotic factor, and as a regulator of the pattern of cytokine secretion (Sprietsma, 1999). Moreover, glutathione is central for antioxidative defense in the lungs, which may be very important in connection with lower respiratory infections including influenza (Cai *et al*., 2003).

11.3 MILK ENZYMES

Endogenous enzymes are milk constituents that originate from four main sources: blood plasma, secretory cell cytoplasm, milk fat globule membrane (MFGM), and somatic cells (leukocytes) (Fox & Kelly, 2006a). Around 70 endogenous enzymes have been identified in normal bovine milk (Fox, 2003). Table 11.2 summarizes the concentrations and activities of some endogenous enzymes present in the milk of several species. Moreover, Table 11.3 presents examples of some of the enzymes that have been well characterized regarding their activity and significance. Additionally, in Table 11.4 some endogenous enzymes that have been suggested to play a key role in the manufacture and/or quality of milk and its derivatives are presented. The best-characterized enzymes in milk include *N*-acetyl-β-D-glucosaminidase, acid phosphatase, alkaline phosphatase, amylase, catalase, γ-glutamyl transferase, glutathione peroxidase, lactoperoxidase, lipoprotein lipase, lysozyme, plasmin, ribonuclease, sulfhydryl oxidase, superoxide dismutase, and xanthine oxidoreductase (Kelly & Fox, 2006). Most of the endogenous enzymes in milk have no obvious physiological role in biosynthesis and secretion of milk (Fox & Kelly, 2006a). Moreover, since these enzymes have no essential beneficial effect on the nutritional or organoleptic attributes of milk, their destruction by heat is one of the purposes of many dairy processes. Besides endogenous enzymes, milk also contains proteases and lipases that are produced by contaminating bacteria during handling and processing. Even when several heat treatment steps are used to prepare milk products, these will not be enough to inactivate all the enzymes and extreme heat treatments will have adverse effects on the products (Chen *et al*., 2003). Proteinases and lipases surviving pasteurization and spray-drying treatments can cause important changes in the functionality and flavor of milk products (Visser, 1981; Renner, 1988; Deeth, 2006).

Table 11.2. Concentrations and activities of some endogenous enzymes in milk from several species.

Enzyme concentration and/or activity	Cow	Human	Goat	Sheep	Buffalo	Camel
Acid phosphatase	0.75 U/mL		1.4 U/mL			
Alkaline phosphatase	1.8–2.5 U/mL		11–13 mg/L		1.2–1.7 U/mL	
Amylase					1.2–1.7 U/mL	
Lactoperoxidase	30 mg/L 1.5–2.7 U/mL	1.5 mg/L 0.06–0.97 U/mL	1.55–4.45 U/mL	0.77–3.46 U/mL	0.2–0.9 U/mL	1.5 U/mL
Lipoprotein lipase	0.5–2.0 mg/mL	4–20 mg/mL				
Lysozyme	10–35 μg/dL	4–40 μg/dL	25 μg/dL	23–50 μg/dL	13–15.2 μg/dL	228–500 μg/dL
Plasmin	0.07–0.3 μg/mL	0.07–0.13 μg/mL				
Ribonuclease	1000–2000 μg/dL	10–20 μg/dL	425 μg/dL			
Sulfhydryl oxidase	33 mg/mL					
Xanthine oxidoreductase	120 μL O_2/h/mL	12 μL O_2/h/mL	19–113 μL O_2/h/mL		120 μL O_2/h/mL	

Sources: based on data from Chen *et al*. (2003), Seifu *et al*. (2005), Fox & Kelly (2006a) and Park & Haenlein (2006).

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Table 11.3. Endogenous bovine milk enzymes.

Enzyme	EC no.	Source	Activity	Optimal pH	Optimal *T* (°C)	Properties	Interest for dairy industry
Plasmin	3.4.21.7	Blood Associated with casein micelle in milk	Serine protease Active on all caseins, particularly on β-casein and α_{s2}-casein	7.5	37	Most plasmin survives pasteurization, though considerable inactivation occurs at higher temperatures Thermal inactivation depends also on the presence of β-lactoglobulin	Cheesemaking properties of milk deteriorate as a result of plasmin activity In the cheese itself, plasmin contributes to primary proteolysis Plasmin and plasminogen have a role in the physical instability or age gelation of UHT milk
Lipoprotein lipase	3.1.1.34	Mammary gland Associated with casein micelle and some in the serum phase	Glycoprotein Liberates fatty acids from the 1 and 3 position in triglycerides, diglycerides and monoglycerides	9.2	37	Heat-labile enzyme Very little activity survives pasteurization Complete thermal inactivation occurs for heat treatment exceeding 75°C for 15 min	Technologically significant enzyme from the viewpoint of milk deterioration Lipolysis leads to the release of free fatty acids, which can result in the development of hydrolytic rancidity
Alkaline phosphatase	3.1.3.1	Mammary gland Associated with phospholipid particles in the milk fat globule membrane	Phosphomonoesterase Active against a wide range of substrates Hydrolyzes most phosphate ester bonds and can dephosphorylate caseins under suitable conditions	9.0–10.5	37	Relatively heat-sensitive Its thermal stability is only slightly higher than that of non-spore-forming pathogenic bacteria present in milk	Its thermal inactivation has been effectively used as a sensitive indicator for adequate pasteurization of milk However, this enzyme can be reactivated on subsequent storage, leading to false alkaline phosphatase-positive test results
Lactoperoxidase	1.11.1.7	Mammary gland Exists primarily in the milk serum	Glycoprotein Catalyzes the oxidation of a donor compound (e.g. aromatic amine, polyphenol, aromatic acid)	8.0	—	Possesses antibacterial activity in the presence of H_2O_2 and thiocyanate via catalysis of the oxidation of thiocyanate to hypothiocyanate Heat-stable enzyme Thermal inactivation for temperatures up to 80°C	Preservation of milk quality Indices of the thermal history of milk Antimicrobial activity Commercial source of enzymes

Sources: based on data from Kelly & McSweeney (2003), Fox & Kelly (2006a) and Huppertz & Kelly (2009).

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Table 11.4. Significance of endogenous bovine milk enzymes for the production and/or quality of milk and milk products.

Product	Enzyme	Significance
Raw milk	Lactoperoxidase	Antimicrobial effect
	Xanthine oxidoreductase	
Pasteurized milk	Plasmin	Possible contribution to instability
	Alkaline phosphatase	Index of processing
UHT milk	Plasmin	Possible contribution to gelation on storage
Cream	Lipase	Can cause rancidity
	Lactoperoxidase	Indicator of heat treatment
Milk powders	Lipase	Can cause rancidity
	Plasmin	Can survive drying and remain active
Yogurt	Lactoperoxidase	Possible inhibition of post-acidification
	Plasmin	Possible effect on gel structure and texture
Fresh cheese	Plasmin	Affects rennet coagulation in milk
Ripened cheese		
General	Plasmin	Contributes to primary proteolysis
	Lipase	Contributes to lipolysis
Swiss	Cathepsin D	May contribute to proteolysis due to inactivation of chymosin
Acid	Cathepsin D	May contribute to proteolysis due to low pH

Sources: adapted from Kelly & Fox (2006), with permission of Elsevier. Additional data based on Kelly *et al*. (2006).

11.3.1 Lactoperoxidase

Lactoperoxidase (LPO; EC 1.11.1.7) belongs to the peroxides family of enzymes and is secreted from mammary, salivary, and other mucosal glands, acting as a natural antibacterial agent. It has the ability to catalyze certain molecules, including the reduction of hydrogen peroxide (Bjorck, 1978). This enzyme catalyzes peroxidation of thiocyanate and some halides (such as iodine and bromium), which ultimately generates products that inhibit and/or kill a range of bacterial species (Kussendrager & van Hooijdonk, 2000; Pruitt, 2003).

Several isozymes of LPO have been reported as a result of the differences in the level of glycosylation and deamination of glutamine (Gln) or asparagine (Asn) (Fox & Kelly, 2006a). The enzyme's molecular mass is 78.0 kDa and its primary structure contains 612 amino acids (Cals *et al*., 1991). The molecule is highly structured, with 65% β-structure, 23% α-helix and 12% unordered structure (Sievers, 1980). LPO binds Ca^{2+}, which has a major effect on its stability, including its heat stability. At a pH below 5.0, the Ca^{2+} is lost, with consequent loss of stability.

During the pasteurization process, LPO is not inactivated, suggesting its stability as a preservative (Table 11.3). This enzyme's biological function has been mainly associated with its antimicrobial activity, and to date there is no report on other direct functions such as immunomodulation or cancer prevention (Rodrigues & Teixeira, 2009).

11.3.2 Catalase

Catalase (H_2O_2:H_2O_2 oxidoreductase; EC 1.11.1.6) catalyzes the decomposition of hydrogen peroxide in water and oxygen, and also oxidizes reducing agents. The catalase activity in milk varies with feed and stage of lactation, and especially during mastitis its level is markedly increased (Johnson, 1974). It has been reported that this endogenous enzyme is concentrated in the cream (specific activity is 12 times higher than in skim milk), thus the MFGM is usually used as the starting material for isolating catalase from milk (Kitchen *et al*., 1970).

Catalase has a molecular mass of 250 kDa (Ito & Akuzawa, 1983a) and three isozymes have been found in the catalase isolated from cream (Ito & Akuzawa, 1983b). Furthermore, catalase is relatively heat labile (Farkye & Imafidon, 1995) and its inactivation has been evaluated as a possible index of thermization of milk (almost completely inactivated by heating at 65°C for 16 s) by Hirvi and Griffiths (1998).

11.3.3 Xanthine oxidoreductase

Xanthine oxidoreductase (XOR; EC 1.13.22; 1.1.1.204) is an endogenous enzyme of milk capable of oxidizing xanthine and hypoxanthine with the concomitant reduction of O_2 to H_2O_2 (Fox & Kelly, 2006a). For its catalytic activity, XOR has been found to require FAD^+ (Massey & Harris, 1997; Harrison, 2004). XOR is concentrated on the MFGM, where it is the second most abundant protein,

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representing 20% of the protein of the MFGM. This enzyme is a dimer with two identical subunits (146 kDa), each containing 1332 amino acid residues in the case of the bovine milk enzyme. Each XOR monomer contains one atom of molybdenum (Mo), one molecule of FAD^+ and two Fe_2S_2 redox centers. NADH acts as a reducing agent.

Milk is a good source of XOR, at least part of which is transported to the mammary gland via the bloodstream. Human milk contains XOR, although its levels vary markedly during lactation. The XOR activity in human milk is low because 95–98% of the enzyme molecules lack Mo (Atmani *et al*., 2004). Also, the level of XOR activity in goat, sheep and buffalo milk is low (Pandya & Khan, 2006). The level of XOR activity in milk can be increased by supplementing the diet with Mo (Fox & Kelly, 2006a).

11.3.4 Proteinases

Proteinases correspond to the group of proteolytic enzymes that act internally on polypeptide chains, rather than cleaving off single amino acids or dipeptides from the ends of polypeptide chains (Chen *et al*., 2003). Furthermore, this group of proteinases is generally classified into four subgroups on the basis of the mechanism of action of the enzyme:

1. Serine proteinases, such as plasmin.
2. Cysteine (or sulfhydryl) proteinases, such as cathepsin B.
3. Aspartic (or acid) proteinases, such as cathepsin.
4. Metalloproteinases, such as thermolysin.

Two particular endogenous milk proteinases have been studied in detail, namely plasmin and cathepsin D (Kelly & Fox, 2006). These proteinases arise from mammary tissue cells, blood plasma, or leukocytes (Fox & Kelly, 2006a).

11.3.4.1 Plasmin

The principal milk endogenous proteinase is plasmin (EC 3.4.21.7) (Table 11.3). The plasmin system has five elements: plasmin, plasmin inhibitors, the inactive zymogen plasminogen, plasminogen activators (PAs), and inhibitors of plasminogen activators (Grufferty & Fox, 1988). This system enters milk from blood and plasmin activity increases during a mastitic infection and in late lactation. Plasmin in milk occurs mainly as the inactive precursor plasminogen (Rollema *et al*., 1981). Plasminogen is activated through proteolysis by PAs, which are serine proteinases (Fang & Sandholm, 1995). In milk, plasminogen, plasmin and PAs are associated with the casein micelles and are concentrated in rennet-coagulated cheese curds and casein, while the inhibitors of PAs and plasmin are soluble in the milk serum (Fox & Kelly, 2006a).

Because of changes in practices in the dairy industry, such as improved bacterial quality, extended storage and the introduction of high-temperature processed milk (plasmin is very heat stable), plasmin has gained an increased significance since its relationship with microbial proteases provides a means to control its levels to benefit the quality of dairy products (Kelly & McSweeney, 2003).

Bovine plasminogen is a single-chain glycoprotein containing 786 amino acid residues, with a molecular mass of 88 kDa. Plasminogen is converted to plasmin by cleavage of the Arg557–Ile558 bond by specific proteinases. Three elements of the plasmin system (plasmin, plasminogen and PAs) have been reported to have very similar and relatively high heat stabilities. For plasmin and plasminogen, this high heat stability is attributed to protection by milk proteins such as casein (Grufferty & Fox, 1988). Plasmin contributes to primary proteolysis in cheese, especially high-cooked varieties in which the coagulant is extensively denatured; it may cause age gelation of ultra high temperature (UHT) sterilized milk; and reduces the yield of cheese and casein owing to the loss of proteose peptones in whey (Rollema *et al*., 1981).

11.3.4.2 Cathepsin D

The second proteinase identified in milk is cathepsin D, which is presumably a lysosomal enzyme (Larsen *et al*., 1996). As with plasmin, cathepsin D is part of a complex system, including inactive precursors (Hurley *et al*., 2000). The major form of cathepsin D in milk is the inactive zymogen, procathepsin D (the proenzyme of cathepsin D), although milk also contains low levels of the mature forms of the enzyme (Larsen *et al*., 2000).

The level of cathepsin D in milk is correlated significantly with the somatic cell count, although it is not clear whether this reflects increased production of cathepsin D and/or increased activation of precursors (Hurley *et al*., 2000). Cathepsin D has a pH optimum of 4 and a molecular mass of 36 kDa and can degrade all milk proteins except β-lactoglobulin (Larsen *et al*., 1996). It is completely inactivated by heat treatment of 70°C for 10 min at pH 4 in acetate buffer and by pasteurization at 65°C for 30 min in skim milk (Chen *et al*., 2003). Because of this relatively low heat stability, even in milk, cathepsin D has not been regarded as an important enzyme in pasteurized milk and milk products.

11.3.5 Lipases and esterases

Lipolytic enzymes can be defined as carboxylesterases that hydrolyze acylglycerols (Beisson *et al*., 2000). Those that hydrolyze acylglycerols comprising C10 fatty acids are the esterases, or carboxylases (EC 3.1.1.1); those that hydrolyze acylglycerols of C10 or greater fatty acids are the

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lipases, or triacylglycerol acylhydrolases (EC 3.1.1.3). Esterases are active in aqueous solutions, while true lipases are more active at lipid–water interfaces rather than in the aqueous phase and most are also capable of hydrolyzing esterase substrates (Chen *et al*., 2003).

Lipoprotein lipase (LPL) accounts for most of the lipolytic activity in bovine milk (Olivecrona *et al*., 2003) and shares about 30% sequence identity with pancreatic lipase, which is regarded as a typical lipase (Fox & Kelly, 2006a) (Table 11.3). LPL is a dimer of glycoprotein chains (two N-linked oligosaccharides), each of 42 kDa, and contains 8.3% carbohydrate (Olivecrona *et al*., 2003). This enzyme is synthesized in mammary gland secretory cells and its level in bovine milk is dependent on the breed, stage of lactation, diet and nutrition, the season, and milk production level (Deeth, 2006).

LPL can form large aggregates, regardless of ionic strength, and yet retain an active conformation (Olivecrona & Bengtsson, 1984). Moreover, this enzyme is relatively unstable to heat. High-temperature short-time (HTST) pasteurization (72°C for 15 s) inactivates most, if not all, of the enzyme in milk (Deeth, 2006), and therefore LPL causes little, if any, lipolysis in pasteurized milk and products derived from pasteurized milk (Chen *et al*., 2003). In most milk samples, LPL causes hydrolytic rancidity only if the MFGM is damaged, for example by agitation, foaming, cooling/warming, freezing or homogenization (Chen *et al*., 2003).

Esterases are distinguished from lipases by their preference for soluble rather than emulsified ester substrates. Milk contains several esterases (Kitchen, 1985; Chen *et al*., 2003), the most significant of which are acylesterases (EC 3.1.1.7), cholinesterase (EC 3.1.1.8), and carboxylesterase (3.1.1.1).

11.3.6 Amylase

Amylase is endogenous in milk and α-amylase is the principal enzyme, with a lesser amount of β-amylase; the enzymes partition mainly into skim milk and whey (Fox & Kelly, 2006a). The α-amylase in milk is similar to salivary amylase. Amylase is quite labile to heat and loss of amylase activity was proposed as a reliable index of the intensity of heat treatment applied to milk. Since bovine milk contains no starch and only low levels of oligosaccharides, the function of amylase in milk is unclear.

11.3.7 Alkaline phosphatase

Alkaline phosphatase (EC 3.1.3.1) is a membrane-bound glycoprotein that is widely distributed in animal tissues and in microorganisms (Table 11.3). It is a very important enzyme in clinical chemistry, its activity in various tissues being an indicator of disease states. Nevertheless, its physiological roles are still unclear.

The alkaline phosphatase activity of bovine milk varies considerably between individuals, and throughout lactation; activity varies inversely with milk yield but is independent of fat content, breed, and feed (Fox & Kelly, 2006b). Alkaline phosphatase is concentrated in cream and is released into buttermilk on churning.

Alkaline phosphatase is a homodimer of two identical subunits, each of molecular mass 85 kDa; it contains four atoms of Zn which are essential for activity and is also activated by Mg^{2+} (Fox & Kelly, 2006b). Alkaline phosphatase is inhibited by metal chelators; the apoenzyme may be reactivated by the addition of one of a number of metals, which is used as the principle of methods to determine very low concentrations of zinc in biological systems. It is also inhibited by inorganic phosphate.

11.3.8 Acid phosphatase

Acid phosphomonoesterase (EC 3.1.3.2) in milk has an optimal pH of 4.0 and is very stable to heating (for complete inactivation, heating at 88°C for 10 min is required). The enzyme is not activated by Mg^{2+} (as is alkaline phosphatase), but it is activated slightly by Mn^{2+} and is very strongly inhibited by fluoride (Fox & Kelly, 2006b).

About 80% of the acid phosphatase in cow milk is found in the skim milk but the specific activity is higher in cream. There is only one isozyme of acid phosphatase in milk that is strongly attached to the MFGM and is not released by non-ionic detergents (Kitchen, 1985). Furthermore, about 40% of the acid phosphatase in skim milk partitioned into the whey on rennet coagulation.

The acid phosphatase isolated from skim milk is a glycoprotein with a molecular mass of 42 kDa and an isoelectric point of 7.9. It is inhibited by many heavy metals, oxidizing agents, orthophosphates, and polyphosphates and is activated by thiol-reducing agents and ascorbic acid; it is not affected by metal chelators (Andrews, 1976). This enzyme contains a high level of basic amino acids and no methionine.

Although acid phosphatase is present in cow milk at a much lower level than alkaline phosphatase, its greater heat stability and lower pH optimum may make it technologically significant. The suitability of acid phosphatase as an indicator enzyme for super-pasteurization of milk has been reported, although it is not as useful as other alternatives (e.g. γ-glutamyl transferase or LPO) (Andrews *et al*., 1987).

11.3.9 Ribonuclease

Ribonuclease (RNase) catalyzes cleavage of the phosphodiester bond between the 50-ribose of a nucleotide and the phosphate group attached to the 30 position of ribose of an adjacent pyrimidine nucleotide, forming a 20,30-cyclic phosphate which is then hydrolyzed to the corresponding

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30-nucleotide phosphate. RNases of various origins and with different biological functions have been characterized. RNase occurs in various tissues and secretions, including milk (Fox & Kelly, 2006b).

RNase in cow milk is optimally active at pH 7.5 and is more heat-stable at acid pH than at pH 7. Little or no RNase activity survives UHT sterilization (121°C for 10 s) but about 60% survives heating at 72°C for 2 min (Meyer *et al*., 1987) or at 80°C for 15 s (Griffiths, 1986). RNase activity in raw or heat-treated milk is stable to repeated freezing and thawing and to frozen storage for at least a year (Meyer *et al*., 1987). It has been suggested that RNase can inhibit bacteriophage, which can inhibit the growth of starter cultures in cheesemaking. Otherwise RNase has no technological significance in milk, while it may have significant biological functions.

11.3.10 *N*-Acetyl-β-D-glucosaminidase

N-Acetyl-β-D-glucosaminidase (NAGase; EC 3.2.1.30) hydrolyzes terminal non-reducing *N*-acetyl-β-D-glucosamine residues from *N*-acetyl-β-D-glucosaminides, including glycoproteins and fragments of chitin. However, NAGase is not specific for *N*-acetyl-β-D-glucosaminides, since it can also hydrolyze *N*-acetyl-β-D-galactosaminides.

NAGase is thought to be a lysosomal enzyme that originates principally from mammary gland epithelial cells and, to a lesser extent, from somatic cells. More than 95% of NAGase in cow milk is in the skim milk. The enzyme is optimally active at 50°C and pH 4.2 (Fox & Kelly, 2006b).

NAGase is inactivated by HTST pasteurization and it has been proposed as a suitable indicator enzyme for assessing heat treatment in the range 65–75°C for 15 s (Andrews *et al*., 1987).

11.3.11 Lysozyme

Lysozyme (EC 3.1.2.17) is a widely distributed enzyme that lyses certain bacteria by hydrolyzing the $\beta(1\rightarrow4)$ linkage between muramic acid and *N*-acetylglucosamine of mucopolysaccharides in the bacterial cell wall. Although lysozyme is a lysosomal enzyme, it is found in soluble form in many body fluids and the lysozyme in milk is usually isolated from whey, indicating that it is in solution, like other lysosomal enzymes such as cathepsin D (Fox & Kelly, 2006b).

The bovine milk lysozyme presents an optimum pH of 6.4, a molecular mass of 18 kDa and its amino acid composition and immunological properties are considerably different from those from other species (e.g., human or equine milk). The amino acid sequence of lysozymes is highly homologous with that of α-lactalbumin. All lysozymes are relatively stable to heat at acid pH values but are relatively labile at pH above 7. More than 75% of the lysozyme activity in bovine milk survives heating at 75°C for 15 min or 80°C for 15 s and therefore it is not affected by HTST pasteurization.

Lysozyme's most probable physiological role is to act as a bactericidal agent. One might expect that, owing to its bactericidal effect, endogenous milk lysozyme would have a beneficial effect on the shelf-life of milk; nevertheless such effects do not appear to have been reported. Exogenous lysozyme may be added to milk for many cheese varieties (e.g., Gouda, Edam, Emmental, Parmigiano Reggiano) as an alternative to KNO_3 to prevent the growth of *Clostridium tyrobutyricum*, which causes late gas blowing and off-flavors.

11.3.12 γ-Glutamyl transferase

γ-Glutamyl transferase (GGT; EC 2.3.2.2) catalyzes the transfer of γ-glutamyl residues from γ-glutamyl-containing peptides. In milk, GGT is found in the membrane material in skim milk (70%) or in the MFGM, from which it can be dissociated by detergents or organic solvents. The enzyme has a molecular mass of 80 kDa and consists of two subunits of 57 and 25 kDa, both of which are glycoproteins (Baumrucker, 1980). The enzyme, which associates strongly, is optimally active at pH 8.5–9 and 45°C and has an isoelectric point of 3.9. It is strongly inhibited by diisopropylfluorophosphate, iodoacetamide and metals (Farkye, 2003). GGT activity in human and bovine milk varies during lactation, being highest in colostrum.

GGT has a role in the regulation of cellular glutathione and may be involved in the transport of amino acids from blood into the mammary gland via the so-called γ-glutamyl cycle and thus may be involved in the biosynthesis of milk proteins (Fox & Kelly, 2006b).

11.3.13 Superoxide dismutase

Superoxide dismutase (SOD; EC 1.15.1.1) scavenges superoxide radicals. The H_2O_2 formed may be reduced to water and oxygen by catalase, peroxidase, or a suitable reducing agent. SOD has been identified in many animal and bacterial cells, its biological function being to protect tissue against free radicals of oxygen in anaerobic systems. There are four isoforms of SOD: Cu/Zn-SOD, extracellular (EC) SOD, Mn-SOD and Fe-SOD. Cu/Zn-SOD is the most common form in mammals and has been isolated from a number of tissues, including bovine erythrocytes. The enzyme, which is very stable in 9 mol/L urea at neutral pH, consists of two identical subunits of molecular mass 16 kDa (153 amino acid residues), held together by one or more disulfide bonds (Hara *et al*., 2003). Mn-SOD and EC SOD are tetrameric enzymes with subunits of molecular mass 20 and 35 kDa, respectively.

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11.3.14 Sulfhydryl oxidase

Sulfhydryl oxidase (EC 1.8.3) catalyzes the oxidation of SH groups of cysteine, glutathione and proteins to disulfides. The enzyme is widely distributed in cell membranes, including those of the mammary gland, kidney, pancreas and intestine. Sulfhydryl oxidase is a glycoprotein (10% carbohydrate) containing 0.5 atoms of Fe per monomer (89 kDa). The enzyme is optimally active at pH 7 and 35°C and is inhibited by metal chelators and SH-blocking reagents.

Sulfhydryl oxidase oxidizes reduced RNase and restores enzymatic activity, suggesting that its physiological function is the formation of specific disulfide bonds during the post-synthesis processing of proteins. Its significance in the dairy industry is its ability to oxidize SH groups exposed and activated during high-temperature processing and which are responsible for the cooked flavor in such products (Swaisgood, 2003). Apparently, oxidation of the SH groups renders the product more stable to lipid oxidation, although SH groups per se are antioxidants.

11.3.15 Aldolase

Aldolase (EC 4.1.3.13) reversibly hydrolyzes fructose 1,6-diphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. It is a key enzyme in the glycolytic pathway. Its presence in cow milk has been reported, and although most (66%) of the aldolase in milk is in the skim milk fraction (Kitchen *et al*., 1970), it is concentrated in the cream (Keenan & Mather, 2006). The aldolase is located in the cytoplasm of the mammary cells, from which the enzyme in milk presumably originates, although some may be from blood. Furthermore, it has been suggested that aldolase plays a role in flavor development in dairy products.

11.3.16 Glutathione peroxidase

Glutathione peroxidase (EC 1.11.1.9) is widespread in the cytoplasm of animal tissues, especially erythrocytes. Its function is to protect the cell against the damaging effects of peroxides, as part of the antioxidative system that includes SOD (Avissar *et al*., 1991). Glutathione peroxidase is a tetrametric protein of four identical subunits (21 kDa), each of which contains one atom of selenium (Se). The molecule has been well characterized, including elucidation of its primary, secondary and tertiary structures (Liu & Luo, 2003).

Glutathione peroxidase has no known enzymatic function in milk, in which it binds 30% of the total Se, an important trace element in the diet. The level of glutathione peroxidase in milk varies with the species and diet (Farkye, 2003).

11.4 MILK HORMONES AND GROWTH FACTORS

A clear division between milk hormones, cytokines and growth factors is currently missing (Gauthier *et al*., 2006). All these molecules are important for the growth, maturation or repair of different cell types in the neonate and/or adult. Moreover, all these growth-promoting factors are signaling molecules released by cells to communicate with each other. Briefly, hormones are substances that are released into the extracellular medium by the cells of a given tissue, to be transported to a new site of action (endocrine function), where they induce a specific response. The distinction between cytokines and growth factors is not straightforward since some growth factors such as transforming growth factor (TGF)-β have also been reported as cytokines by many authors (Gauthier *et al*., 2006). Cytokines are proteins or glycoproteins produced by many cell types that have profound bioactive effects on other cells within a short distance at low concentrations (Playford *et al*., 2004). As a result, cytokine effects are local and these agents are involved in autocrine or paracrine functions. Examples of cytokines include the interleukin (IL) series, tumor necrosis factor (TNF) and interferon (IFN). Growth factors are proteins or polypeptides that bind to specific receptors triggering intracellular secondary messengers, ultimately resulting in cellular proliferation and/or differentiation (Michaelidou & Steijns, 2006).

Milk contains more than 50 growth factors and hormones, their concentrations being much lower than those of immunoglobulins or lactoferrin (Michaelidou & Steijns, 2006). Whereas bovine colostrum may contain high levels of cytokines such as IL-1β, IL-6, TNF-α, INF-γ, and IL-1 receptor antagonist, their levels in mature milk are remarkably lower (Hagiwara *et al*., 2000). Whey has also been identified as a source of growth factors with potent and proven bioactivity (Smithers, 2004). Examples of growth factors identified in milk include insulin-like growth factor (IGF)-I and IGF-II, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF)-β, and betacellulin (BTC) (Rogers *et al*., 1995; Pakkanen & Aalto, 1997; Dunbar *et al*., 1999; Elfstrand *et al*., 2002). Since these compounds are potent growth stimulants and mediators for a range of mammalian cells, they constitute potential bioactive ingredients in a range of biomedical and functional foods (Smithers, 2004). Milk derived TGF-β might be exploited in functional foods for the infant or during therapies for specific intestinal diseases or cancers (Donnet-Hughes *et al*., 2000).

Milk-derived products are already in clinical use for the treatment of inflammatory bowel disease. Casein-based enteral feeds are used for the treatment of Crohn disease (Beattie *et al*., 1998; Donnet-Hughes *et al*., 2000) and their

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Table 11.5. Bovine milk hormones.

Hormone	Concentration range (ng/mL)
Estrogen	5×10^{-3} to 10×10^{-3}
Progesterone	2–20
Glucocorticoids	0–50
Prolactin	5–200
Growth hormone	0–1
Somatostatin	10–30
Parathyroid hormone-related protein	40–100
Insulin	5–40
Calcitonin	700
Bombesin	0.25–450
Melatonin	5×10^{-3} to 25×10^{-3}

Source: adapted from Jouan *et al*. (2006), with permission of Elsevier.

efficacy might be due to the presence of growth factors (Playford *et al*., 2000). Also, results from animal and human trials with a growth factor extract from Cheddar cheese whey targeting oral mucositis and chronic ulcers were very promising (Smithers, 2004). Dairy-derived preparations appear to be an attractive therapeutic option (Regester & Belford, 1999) because they contain many different growth factors in a formulation that provides additive or synergistic activity and inherent protection against proteolytic digestion. Besides, such preparations have the advantage of being perceived as "natural" products, which may result in greater patient acceptance and compliance (Playford *et al*., 2000).

11.4.1 Hormones

Milk contains several hormones at trace levels that have limited nutritive or diagnostic value. However, many studies regarding the physiological roles of hormones in human and bovine milk have been conducted (Koldovsky & Thornburg, 1987; Grosvenor *et al*., 1993). Hormones in milk originate from the blood and are secreted in milk through active transport within the mammary gland. Also, some hormones can be synthesized by the mammary gland and excreted to milk. Table 11.5 summarizes the main hormones that have been identified in bovine milk, as well as the concentration ranges (Jouan *et al*., 2006). Most hormones reported in milk are classified into four main groups: gonadal, adrenal, pituitary, and hypothalamic hormones. Other molecules, such as proteins related to parathyroid hormone, have also been reported as hormones.

11.4.1.1 Gonadal hormones

The gonadal hormone group includes estrogens, progesterone and androgens, the androgens being the least studied hormones. Since the amount of hormones in milk and milk products is very low, their accurate quantification remains a challenge. Several techniques have been proposed for this purpose, such as colorimetry, spectrofluorometry, gas chromatography and high-pressure chromatography, and radioimmunoassay. The concentrations of estrogens, namely 17β-estradiol, estrone and estriol, in milk and several dairy products have been reported (Wolford & Argoudelis, 1979). The cow milk fat fraction was found to contain 65% of 17β-estradiol and 80% of estrone. The occurrence of estrogens in both butter and skim milk clearly indicates a distribution of those steroids between the lipid and serum phases of milk. Estrone was found to be the predominant estrogen in milk, and it is well known that estrogen concentrations are related to gestation and reproductive cycle. Regarding progesterone levels in milk, they have been found to be related to pregnancy and parturition (Comin *et al*., 2005). In cow milk, progesterone concentrations have been determined by gas chromatography and found to be around 0.3–0.4 pg/mL (Darling *et al*., 1974). The progesterone concentrations were found to be higher in cream than in skim milk. Ginther *et al*. (1976) determined the progesterone content of several dairy products from the cow, namely 11.4 ng/mL in whole milk, 4.7 ng/mL in skim milk, and 58.8 ng/mL in cream.

11.4.1.2 Adrenal gland hormones

Adrenal gland hormones comprise essentially the glucocorticoids, which have been identified in bovine milk in concentrations between 0.7 and 1.4 ng/mL (Gwazdauskas *et al*., 1977). No remarkable differences have been found between whole and skim milk. Cortisol and corticosterone are the main glucocorticoids in blood plasma of cows (Tucker & Schwalm, 1977). During lactation, the glucocorticoid concentrations in milk represent only 4% of the blood plasma concentrations, suggesting that only a small amount is transferred to the milk. Glucocorticoids are not concentrated in cream, as is the case of estrogens. In bovine milk, corticosteroids are equally distributed between caseins and whey protein fractions. Glucocorticoids possibly act in conjunction with other hormones to maintain lactation and their effects are mediated by specific receptors. Furthermore, there seems to be a reduction in glucose uptake by the mammary gland due to the presence of glucocorticoids, suggesting that milk production is regulated by these hormones.

11.4.1.3 Pituitary hormones

Two hormones have been identified in the category of pituitary hormones, namely prolactin and growth hormone. Prolactin concentrations of 5–200 ng/mL have been detected by radioimmunoassay in bovine milk (Malven &

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McMurtry, 1974). It has been suggested that prolactin concentrations vary seasonally, being higher in the summer, and a direct effect of storage temperature of the milk appears to affect this hormone concentration. Lower temperatures have a negative impact on prolactin concentrations. Kacsoh *et al*. (1991) reported that part of the prolactin content of milk is associated with the milk fat globules, so that 60% of the prolactin is removed from milk during the centrifugal separation of fat. This hormone is thought to originate from blood plasma and its biological functions are not well known. Growth hormone or somatotropin has been detected in milk at concentrations lower than 1 ng/mL (Torkelson, 1987). Regarding its biological functions, somatotropin is thought to be acting on the mammary gland by means of specific receptors. Furthermore, this hormone was also found to increase the concentration of IGF-I in epithelial cells of the mammary gland of lactating cows (Glimm *et al*., 1988).

11.4.1.4 Hypothalamic hormones

The group classified as hypothalamic hormones includes the gonadotropin-releasing hormone, luteinizing hormone-releasing hormone, thyrotropin-releasing hormone and somatostatin. All these hormones have been detected and quantified in bovine milk mainly using radioimmunoassay. Gonadotropin-releasing hormone content in milk is five to six times greater than in blood plasma (Baram *et al*., 1977). The hormone might be from an extra-hypothalamic origin but it is more likely to come from active transport by the mammary gland. Amarant *et al*. (1982) determined the luteinizing hormone-releasing hormone content in milk and colostrums. This hormone may originate from the blood and be concentrated in the mammary gland by an active process or be of extra-hypothalamic origin. The same authors also measured thyrotropin-releasing hormone in milk and colostrum. As for the other hypothalamic hormones, their origin in milk is unclear. The occurrence of somatostatin in bovine milk has been demonstrated by enzyme immunoassay on fat and casein-free milk (Takeyama *et al*., 1990). Its concentrations in milk vary between 10 and 30 pmol/L and it does not seem to be affected by parturition.

11.4.1.5 Other hormones

As previously mentioned, other molecules, such as proteins related to parathyroid hormone, insulin, calcitonin, bombesin, erythropoietin and melatonin, have also been identified in milk although they are less known and characterized. Many authors have mentioned that parathyroid hormone-related protein is present in bovine milk (Budayr *et al*., 1989; Ratcliffe *et al*., 1990). Curiously the concentrations of this hormone in both fresh and pasteurized milk have been found to be similar, suggesting that this hormone is heat-stable. The physiological functions of this hormone have not been clearly established. Produced by the mammary gland, this hormone might be involved in the transport of calcium from blood plasma to milk. Regarding insulin, its concentrations in milk have been found to vary during the prepartum and postpartum periods and after parturition (Malven, 1977). In colostrum its content is between 0.67 and 5.0 nmol/L, which is 100-fold higher than the concentration in blood plasma (Ballard *et al*., 1982). Moreover, calcitonin concentrations in human milk have been estimated at 700 ng/mL and this hormone has been found to inhibit the liberation of prolactin (Koldovsky, 1989). As for bombesin, it is known to influence gastric hormonal secretions following ingestion (Lazarus *et al*., 1986). Satiety, blood sugar concentrations, gut acidity, and the concentrations of some gastrointestinal hormones are known to be influenced by bombesin. Bombesin has been found in human milk, bovine milk, milk powder and whey (Koldovsky, 1989). Furthermore, no analytical data regarding the erythropoietin content of bovine milk are available, although this hormone has been identified in human milk (Grosvenor *et al*., 1993). Finally, melatonin is a hormone synthesized by the pineal gland in a diurnal pattern reflecting photoperiodicity. Melatonin has been found in human, bovine and goat milk (Eriksson *et al*., 1998; Valtonen *et al*., 2003) at a low concentration.

11.4.2 Growth factors

The most abundant growth factors in bovine milk and colostrum are IGF-I, TGF-β2, some members of the zepidermal growth factor (EGF) family, and FGF-2 (Grosvenor *et al*., 1993; Pakkanen & Aalto, 1997). Table 11.6 summarizes the experimental data available on the content of some of these growth factors in bovine milk. The concentrations found in colostrum are generally higher than those in milk, except for BTC where the levels appear to be equivalent (Pouliot & Gauthier, 2006). Quantitatively, the relative concentrations of growth factors in milk are as follows: IGF-I > TGF-β2 > EGF ≈ IGF-II > bFGF.

The main biological functions of milk growth factors have been extensively reviewed by Gauthier and *et al*. (2006). Briefly, EGF and BTC are members of the EGF family that have been detected in milk products in sufficient amount to induce physiological effects (Dunbar & Goddard, 2000). They stimulate the proliferation of epidermal, epithelial and embryonic cells, inhibit the secretion of gastric acid, and promote wound healing and bone resorption.

The TGF-β family comprises multifunctional growth and differentiation factors that act on most cell types with activities dependent on the cell type, stage of proliferation and environment (Massague, 1990), thus playing an important

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Table 11.6. Bovine milk growth factors.

Growth factor	Concentration (ng/mL)	Source	Primary activity	Amino acid residues	Molecular mass (g/mol)	pI
EGF	<2.0	Wide range of tissues and body fluids	Stimulates proliferation of epidermal, epithelial and embryonic cells Inhibits secretion of gastric acid Promotes wound healing and bone resorption	53	6000	4.8
BTC	1.9			80	22 000	7.7
IGF-I	30.4	Primarily the liver	Stimulates proliferation of many cell types	70	7650	7.8
IGF-II	50–100	Variety of cells	IGF-I is a stronger mitogen than IGF-II which stimulates primarily cells of fetal origin Influences the differentiation of some cells Causes hypoglycemia, improvement of nitrogen balance, lowering of cholesterol and potassium, and improvement in renal function	67	7530	6.5
TGF-β2	13–71	Platelets and many other cells	Stimulates growth of cells, especially in connective tissue Inhibits other cells, such as lymphocytes and epithelial cells Important role in embryogenesis, wound healing, formation of bone and cartilage, and control of the immune system	425	25 000	7.7
PDGF	NA	Platelets and many other cells	Plays a role in embryonic development, proliferation of cells of mesenchymal origin, migration, angiogenesis and wound healing	250–300	30 000	9.6
FGF2	0.5–1	Wide range of cells	Important role in proliferation, differentiation and survival of many cell types Involved in angiogenesis, wound healing and hematopoiesis	146	16 400	9.6

EGF, epidermal growth factor; BTC, betacellulin; IGF, insulin-like growth factor; TGF, transforming growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; NA, not available.

Sources: based on data from Grosvenor *et al*. (1993) and Gauthier *et al*. (2006).

role in embryogenesis, tissue repair, formation of bone and cartilage, and in control of the immune system. TGF-β2 is the predominant form of the TGF family members found in milk products, and although its physiological function is unknown some authors have suggested that it could be a mediator of mucosal immunity or gut epithelial differentiation in neonates (Cox & Burk, 1991; Jin *et al*. 1991). TGF-β stimulates the proliferation of some cells, especially in connective tissue, whereas they act as growth inhibitors of some other cells, such as lymphocytes and epithelial cells.

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IGF-I and IGF-II stimulate the proliferation of many cell types (Jones & Clemmons, 1995). IGF-I stimulates cellular growth, development and differentiation. Furthermore, IGF-I stimulates glucose uptake and the synthesis of glycogen. Administration of IGFs to humans causes hypoglycemia (Guler *et al*., 1987), improvement in nitrogen balance (Clemmons *et al*., 1992), lowering of cholesterol and potassium (Moscatelli, 1987), and improvement in renal function (Guler *et al*., 1989; Hirschberg *et al*., 1993).

FGF-2 can exert multiple functions on a variety of cells. It has been reported to stimulate proliferation, migration and differentiation of endothelial cells, fibroblasts and epithelial cells (Chen *et al*., 2004). This growth factor also promotes angiogenesis, normal wound healing, tissue development, hematopoiesis, and the synthesis of collagen and fibronectin. It has been suggested that FGF-2 in milk might be bound to the heparan sulfate proteoglycan in the MFGM (Hironaka *et al*., 1997).

11.5 MILK ORGANIC ACIDS

Most work that has been recently conducted has focused on the presence of fatty acids in milk, for example CLA, linoleic acid, myristic, plamitic, butyric and stearic (Mansbridge & Blake, 1997; Parodi, 1999), and less attention has been given to the organic acids (e.g., citric, lactic, acetic) (Mullin & Emmons, 1997). Citric acid is the predominant organic acid in milk. During storage it disappears rapidly as a result of the action of bacteria. Other acids (lactic, acetic) are degradation products of lactose. The occurrence of orotic acid, an intermediary product in the biosynthesis of pyrimidine nucleotides, is specific for milk. Orotic acid, as well as total creatinine and uric acid, are suitable indicators for the determination of the proportion of milk in foods. The contents of orotic acid, creatinine and uric acid in milk vary among the different species. For example, the content in orotic acid is low in buffalo milk (19.6 µg/dL) as compared to cow milk (52.6 µg/dL), while creatinine is higher (246 mg/dL vs. 167 mg/dL) and uric acid is the same (0.26 mg/dL) (Park & Haenlein, 2006).

Raw milk produced under normal conditions develops acidity. It has long been recognized that highly acid milk does not putrefy. Therefore, allowing milk to develop acidity naturally preserves the other milk constituents. Fresh bovine milk is particularly suitable as a fermentation substrate for most microorganisms, since it contains 5% lactose, 3.3% protein, has a water activity near 1.0, and a pH of 6.7. Although milk from other species present different compositions of protein and lactose, they are also suitable for microbial growth (Park & Haenlein, 2006). Milk samples from normal healthy mammary glands contain many strains of bacteria (Haug *et al*., 2007).

Bacteria in milk are responsible for acid development. They produce acid by the anaerobic breakdown of lactose to lactic acid and other organic acids. A number of sugar fermentations depending on the microorganism involved and the end products have been reported in milk. For example, if only streptococci and lactobacilli are present, then the end product will be lactic acid. The lactic acid fermentation is the most important one in milk and is central to many processes (Mullin & Emmons, 1997). LAB are saccharolytic and fermentative, and therefore are ideally suited for growth in milk. In general, they will outcompete other microorganisms for lactose, and due to acidification they will produce an unfriendly environment for competitors. Therefore, when properly made, cultured dairy products (fermented milks) have long shelf-lives and although growth of acid-tolerant yeast and molds is possible, growth of pathogens rarely occurs.

Propionibacteria will ferment lactose, producing lactic acid, propionic acid, acetic acid, and carbon dioxide. Propionic fermentation is a mixed-acid fermentation and is used in the manufacture of Swiss cheese varieties. Yeasts, such as *Candida* and *Torula*, will produce ethanol and carbon dioxide. Alcohol fermentation can be used to prepare certain fermented milks and also to make ethanol from whey. On the other hand, coliform fermentation can also occur with the production of lactic acid, acetic acid, ethanol, carbon dioxide, and hydrogen. This type of fermentation is an example of undesirable spoilage fermentation. Large numbers of coliform bacteria in milk indicates poor hygiene. The coliform fermentation disrupts lactic acid fermentation and also causes spoilage in cheese. The type of fermentation obtained will depend on the numbers and types of bacteria in milk, storage temperature, and the presence or absence of inhibitory substances. Different bacteria may be used for fermentation, giving products special flavors and aromas, and with several potential health beneficial metabolites (Rossland *et al*., 2005). Fermented milks (buttermilk, kefir, coumis, aigar, among others) are nutritious foods and many have been reported as possessing medicinal properties (De Vrese *et al*., 2001; Branca & Rossi, 2002; Sanggaard *et al*., 2004; Vinderola *et al*., 2005).

Organic acids play an important role in fermented milks and cheese, and can be changed due to technical options in the process. Park and Guo (2006) reviewed the organic acid composition of several types of goat cheese depending on the processing. For example, for plain soft goat cheese they found differences in tartaric, formic and uric acid contents between fresh and frozen-thawed treatments. Freezing caused an increase in formic and uric acids and a decrease in tartaric acid. These changes in organic acid contents of plain soft goat cheese are in contrast with the results

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reported by Califano and Bevilacqua (1999), who found no significant effect of freezing on the variations in organic acid contents of cow milk Mozzarella cheese. Also, Park and Guo (2006) reported that aging (refrigerated storage at 4°C for 28 days) of plain soft goat cheese promoted changes in only three acids (orotic, malic and butyric). In Monterey Jack cheese, frozen and thawed cheese had higher acetic, butyric, citric, malic, propionic, and pyruvic acids compared to the unfrozen control cheese. The levels of acetic, butyric, malic, and orotic acids were elevated by aging time.

11.6 FUTURE PERSPECTIVES AND CONCERNS

Milk and milk products are commonly used in many food regimens, and although the operations for their processing are known and optimized, many challenges persist (Villamiel *et al*., 2009). Within modern societies milk has to be treated in different ways to be kept for several days. This processing includes steps that may be of concern. In fresh milk each lipid globule is surrounded by an apical plasma membrane from the mammary epithelial cell. It is not known, although debated, whether the process of milk homogenization, when the fat globules with their globule membrane are broken up into many new small lipid droplets with just a small fragment of the originating membrane, might have health implications (Haug *et al*., 2007). Additionally, proteins and peptides are heat sensitive, and their bioactivity may be reduced by pasteurization of milk. Heating of milk may also result in the formation of potentially harmful new products, for example reaction products between carbohydrates in milk with proteins (Lund *et al*., 2005). Also, the amount of some vitamins and antioxidants will be reduced by heating. Glutathione may easily be destroyed during storage (Ankrah *et al*., 2000).

Therefore, the dairy industry has to deal with the important challenge of treating milk in such a way that preserves its vitamins, proteins and peptides. Most of the new technologies currently used are not completely new and they have been explored in the past but with limited success. However, the technical-scientific progress together with consumer demands for minimally processed foods has led to their renaissance. Some dairy industries currently filter milk using membrane processes instead of using pasteurization, and the use of non-thermal processing technologies may yield health benefits (Haug *et al*., 2007). New technologies used in milk processing for the inactivation of microorganisms and enzymes involve microwaves, high pressure, pulsed electric fields, microfiltration, innovative steam injection systems and combined technologies (Villamiel *et al*., 2009).

Regarding novel production methods, it is important to mention that systems, synthetic and metabolic engineering will be in the near future key players in the development of improved and safe microbial cell factories that will enable the production of specific enzymes, vitamins or minor components (Brenner *et al*., 2008; Park & Lee, 2008). Using these tools it will be possible to produce higher amounts of such components, produce them in such a way that recovery is easier and produce differentiated compounds, thus revolutionizing the field of functional foods.

On the other hand, new perspectives also involve the development of novel formulations targeting specific consumer groups, such as newborns, the elderly, diabetics, among others. Nowadays, the use of minor milk components to enrich food products and to develop new ones holds much promise for the emergent fields of personalized nutrition and functional foods (Stover & Garza, 2002; Kaput, 2008; Kussmann & Fay, 2008). Nevertheless, it is extremely important that the benefits claimed for such components are proved using living systems. There is still a dearth of knowledge on the biological activity of the minor milk constituents and new methodologies ought to be developed. Also, the study of the mechanisms of interaction of such components with cell receptors and/or specific genes is still limited and the omics techniques can be very useful for gathering this sort of information (van Ommen & Stierum, 2002; Muller & Kersten, 2003; Ferguson, 2006).

Several challenges and concerns regarding the potential use and understanding of minor milk constituents, enzymes, hormones, growth factors, and organic acids still need to be addressed. Some examples include technological concerns, such as the impact of milk enzymes on the quality of fermented milks; the impact of technological developments in thermal processing on milk constituents, specifically on enzymes, relative to the sensory quality of milk; and the impact of membrane processes on enzyme activities in dairy products. On the other hand, interesting challenges ought to be pursued, such as those related to the commercial availability of certain milk enzymes which have not found widespread application in industry; the potential significance of enzymes and other minor constituents from colostrum; the potential nutraceutical significance of such milk constituents; and the relationship between some enzyme activities and the sensory quality of stored milk and means to control the activity of some enzymes.

Finally, as milk composition varies broadly with several factors such as genetics (e.g., species and breed), stage of lactation, health status, and environmental factors (e.g., feed, climate and method of milking), manipulating these factors constitutes a "natural" way of changing the content of minor constituents in milk. Genetic manipulation of livestock has been limited to the permanent addition of

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genes of clinical interest. Nevertheless, researchers have been exploring the utility of genetically engineered cattle as a means of altering milk composition to improve the functional properties of milk, increasing marketability (Karatzas & Turner, 1997). Improvements would include increasing the concentration of valuable components in milk (e.g., casein), removing undesirable components (e.g., lactose), or altering composition to resemble that of human milk as a means of improving human neonatal nutrition. On the other hand, milk composition can be altered by nutritional management or through the exploitation of naturally occurring genetic variation among cattle (crossbreeding or selection). Additionally, on-farm methods including rumen modification, trait selection and rations can be used to change milk composition (Knowles *et al*., 2006; Haenlein & Anke, 2011).

Through these methods it has been possible to increase concentrations of calcium, selenium, iodine, iron and cobalt/vitamin B_{12} in milk. For example, the CLA content in milk fat can be modified by feeding that influences the pattern of fat precursors the mammary gland removes from blood for fat synthesis (Mel'uchová *et al*., 2008). Variation in CLA content in milk of ruminants appears to be minimally influenced by the stage of lactation, parity and breed (Sanz Sampelayo *et al*., 2007). Nevertheless, diet is the most important factor influencing milk CLA concentration. The CLA concentration in milk is higher in pasture-raised animals than in those fed with dry diets, and it decreases with increasing growth stage of forage or maturity. Off-farm alternative approaches for manipulating milk composition might be less desirable. For instance, fortification at the processor with trace elements and vitamins by way of post-harvest or supplements is common, and usually inexpensive, but many consumers prefer not to consume these additives (Cox, 2008); some legal definitions of fresh milk preclude addition of fortificants; and some markets permit only "unadulterated" foods (e.g., exported infant formula). Regardless of method, manipulations will be appropriate only if they suit typical farming practice and do not perturb other product qualities such as safety, shelf-life, texture or taste.

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12
Lactose Intolerance

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12.1 INTRODUCTION

Lactose intolerance is the inability to digest lactose into its constituents, glucose and galactose, because of low levels of lactase enzyme in the brush border of the duodenum (Rusynyk & Still, 2001). Lactose intolerance is defined as a metabolic disorder when people are unable to digest significant amounts of lactose due to the genetically insufficient production of the lactase enzyme (β-galactosidase) (Suchy *et al*., 2010). Therefore, lactose intolerance is caused by inadequate production of lactase and therefore a condition called lactose malabsorption is the very common characteristic of intestinal lactase deficiency. This chapter reviews the literature on types of lactose intolerance, its symptoms, the prevalence, and dietary approaches to alleviate lactose intolerance with special emphasis on fermented foods and probiotics.

12.1.1 Lactose and lactase

Lactose (β-galactose-1,4-glucose; Fig. 12.1), the major carbohydrate in human and animal milk, is a disaccharide sugar composed of the monosaccharides glucose and galactose (Semenza *et al*., 2001; Harrington & Mayberry, 2008). Since intestines can absorb only monosaccharides, this malabsorption of lactose leads to the condition called lactose intolerance. Lactase, an enzyme (β-galactosidase) found in the lining of the small intestine, acts as a catalyst in cleaving of lactose into the easily digestible glucose and galactose.

In the human body glucose acts as a source of energy and galactose becomes a part of glycolipids and glycoproteins. In the small intestine, lactase activity is high close to the ileum and very low in the first portion of the duodenum and in the terminal part of the ileum (Swallow *et al*., 2001). Lactase splits and hydrolyzes dietary lactose into glucose and galactose for transport across the cell membrane (Suchy *et al*., 2010). People with lactose intolerance are unable to digest significant amounts of lactose because expression of the lactase gene in the cells lining the small intestine produces insufficient quantities of the enzyme (Suarez *et al*., 1995). The lactase gene (*LCT*) encodes lactase, also called lactase-phlorizin hydrolase (LPH), and this possesses lactase and phlorizin hydrolase activity (Torun *et al*., 1979). LPH, the main intestinal lactase, is an essential glycoprotein of the brush border of the upper small intestine (Swagerty *et al*., 2002). Lactase is synthesized as a pro-polypeptide that is glycosylated and proteolytically cleaved inside the cell to form the mature enzyme. Later, active LPH enzyme is transferred to the outer surface of the brush border, showing high activity in the jejunum (Swagerty *et al*., 2002).

In addition to hydrolyzing lactose into glucose and galactose, lactase also cleaves cellobiose, cellotriose, cellotetrose, and to a certain extent cellulose. The phlorizin hydrolase activity breaks β-glycosides with a large hydrophobic alkyl chain (galactosyl- and glycosyl-*h*-ceramides, phlorizin) (Torun *et al*., 1979). Various roles of the phlorizin site are useful in humans following the usual decline in enzyme expression after weaning from breast milk and thus enzyme activity is retained even after the weaning period (Lomer *et al*., 2008). Although lactose is not a key nutritional component for adults, it is the main source of energy during the first year of a human's life, providing almost half

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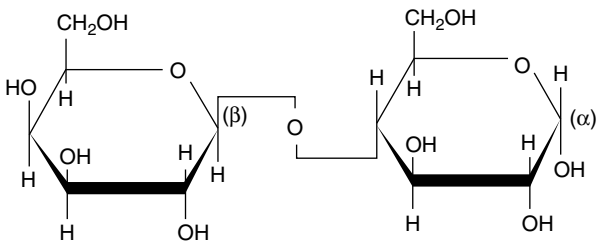


Figure 12.1. α-Lactose (4-*O*-β-D-galactosylpyranosyl-α-D-glucose). Based on Schaafsma (2008).

of the total energy requirement (Vesa *et al*., 2000). Lactose intolerance is not common in those parts of the world where milk is not a staple diet. However, if people from these regions migrate to Europe or North America, the problem arises because of difficulties in ingesting lactose (Fuller, 1991). After the weaning period, lactase activity decreases in most mammals, but in some human ethnic groups such as white western Europeans, lactase activity continues to exist into adult life enabling the digestion of large quantities of dietary lactose (Troelsen, 2005).

All vertebrates have the phlorizin hydrolase activity, whereas the lactase activity has only been found in mammals (Leese & Semenza, 1973). Lactose is present only in mammalian milk, with concentrations of about 7.2 g/dL in mature human milk and 4.7 g/dL in cow milk (Miller *et al*., 2006; Lomer *et al*., 2008). Other non-dairy products that contain lactose include instant breakfast mixes, shakes, coffee whiteners, baby cereals, cake mixes, sausage, mayonnaise, frankfurters, ready to eat foods, and processed foods (Montes & Perman, 1991; Hertzler *et al*., 1996; Cox, 2003; Harrington & Mayberry, 2008). Lactose can be obtained in more or less pure form from milk and whey and can be used as an ingredient in feed, food and pharmaceutical preparations (Schaafsma, 2008). Lactose has several applications in the food industry, including the manufacture of candy, confections, pancakes, waffles and pastries, mainly because of its limited sweetness, solubility, crystallization, and browning properties, and it also provides better texture and binds water and color (van Griethuysen-Dilber *et al*., 1988).

Lactose has several nutritional benefits. The lactose derivatives lactulose, lactitol and galacto-oligosaccharides have applications in foods and pharmaceutical preparations as prebiotics to promote gut health. Similarly, compounds such as tagatose and lactobionic acid have potential applications as bioactive ingredient in foods (Schaafsma, 2008). It is a suitable carbohydrate in infant formula due to its lower sweetening power that may help to prevent the development of a taste preference for sweet foods in later life. Since the glycemic index (GI) of lactose is relatively small, lactose can have benefits for persons who are susceptible to hyperglycemia (Schaafsma, 2008). Lactose also has an effect on mineral absorption. Abrams *et al*. (2002) reported that a lactose-containing infant formula absorbed 10.3% more calcium compared with a lactose-free formula.

Table 12.1. Lactose content of dairy products.

Product	Lactose (g)
Milk (1 cup, 240 mL)	
3.5% fat	9–12
2% fat	9–13
1% fat	12–13
Acidophilus milk	11
Evaporated	24–28
Goat milk	11–12
Sweetened condensed	31–50
Lactaid (lactose-reduced low-fat milk)	3
Yogurt, low-fat (1 cup, 240 mL)	4–17
Cheese (1 oz, 28 g)	
American, pasteurized, processed	0.5–4
Cheddar, sharp	0.4–0.6
Cottage (½ cup, 113 g)	0.7–4
Cream	0.1–0.8
Mozzarella, part skim, low moisture	0.08–0.9
Ricotta (½ cup, 113 g)	0.3–6
Swiss	0.5–1
Butter (1 pat, 9 g)	0.04–0.05
Cream (1 tbsp, 15 mL)	
Light	0.6
Sour	0.4–0.5
Whipping	0.4–0.5
Ice cream (½ cup, 15 g)	2–6
Ice milk (½ cup, 120 mL)	5

Source: based on data from Miller *et al*. (2006).

People with very low tolerance for lactose should know about the many food products other than the dairy products that may contain small amounts of lactose. However, lactose and its derivatives have several nutritional benefits mainly in the promotion of gut health. If taken in moderate dosages and distributed over meals, lactose may act as a prebiotic in lactose-deficient populations (Schaafsma, 2008). Table 12.1 shows the amount of lactose present in different dairy products. For comparison purposes, the lactose content in milk of other mammalian species is presented in Table 12.2. There is a common myth that goat milk is lactose-free (Lomer *et al*., 2008). In fact, all the principal dairy animals (cow, goat, and buffalo) contain the same amount of lactose (4.6–4.8 g/100 g). Therefore, people with lactose intolerance cannot tolerate milk from these major sources.

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Table 12.2. Lactose content of milk from different mammalian species (per 100 g fresh milk).

Species	Lactose (g)	Fat (g)	Protein (g)
Cow	4.6	3.7	3.2
Donkey	6.1	0.6	1.9
Elephant	5.3	5	4
Goat	4.7	3.8	2.9
Human	7	4.2	1.1
Monkey, rhesus	7	4	1.6
Mouse	3	13.1	9
Seal	0.1	49.4	10.2
Water buffalo	4.8	9	4.1
Whale	1.3	42.3	10.9

Source: based on data from Webb *et al*. (1974).

Table 12.3. Types of lactose intolerance.

Types	Pathogenesis
Primary	Genetically predetermined reduction of enzyme activity during childhood or adolescence
Secondary	Temporary inability to digest lactose caused by any condition that leads to injury of the intestinal mucosa or to reductions of the functional mucosal surface area
Congenital	Enzyme activity absent from time of birth

Source: based on data from Rusynyk & Still (2001).

12.1.2 Types of lactose intolerance

Three basic types of lactose intolerance or low lactase activity have been defined and are listed in Table 12.3 (Rusynyk & Still, 2001).

1. *Primary lactose intolerance* is genetically determined and is the most common type in which a low level of lactase develops after weaning (Greenberger & Isselbacher, 1998). This lactose intolerance may not become clinically evident until puberty or late adolescence (Escher *et al*., 1992; Lloyd *et al*., 1992). Lactase levels start to decline by 70–90% by early childhood and continue declining throughout life (Harrington & Mayberry, 2008). Therefore it is also referred to as adult-type hypolactasia, lactase non-persistence, or hereditary lactase deficiency (Heyman, 2006). Although the molecular mechanism causing this decline in lactase is not fully understood, reduced synthesis of the precursor protein in the epithelial cells is believed to be associated with the reduction in lactase activity (Cox, 2003).
2. *Secondary lactose intolerance* occurs as a result of disease, surgery, radiation, or medications that may cause damage to the intestinal mucosa (National Dairy Council, 1978; National Medical Association, 2009). It is attributable to an inability to digest lactose caused by any condition that leads to injury of the intestinal mucosa (acute gastroenteritis, persistent diarrhea, small bowel overgrowth, cancer chemotherapy) or to reductions in the functional mucosal surface area (Rusynyk & Still, 2001). Secondary lactose intolerance may last only for a short time after infective gastroenteritis, and may cause mucosal disease, such as in celiac disease and Crohn disease (Newcomer & McGill, 1984; Cox, 2003). This form of lactose intolerance tends to be transient, depending on the nature of the primary disorder and can be present at any age but is mostly common in infancy (Newcomer & McGill, 1984; Rusynyk & Still, 2001; Heyman, 2006).
3. *Congenital lactose intolerance* is an extremely rare condition where there is complete absence of the enzyme lactase. This is usually visible in the first week of life (National Dairy Council, 1978; Newcomer & McGill, 1984) and is characterized by failure to thrive and infantile diarrhea from the first exposure to breast milk (Lomer *et al*., 2008). As congenital lactose intolerance remains lifelong, this leads to lactosuria because of abnormal absorption of lactose and thereafter renal tubular acidosis, aminoaciduria, vomiting, and failure to thrive (Cox, 2003). If the infant is not fed a lactose-free diet, diarrhea can lead to dehydration and can be fatal (Newcomer & McGill, 1984; Saavedra & Perman, 1989).

Numerous terminologies, such as lactose deficiency, lactase non-persistence, lactose malabsorption, lactose maldigestion, milk allergy and lactose intolerance, to describe lactose absorption have been used indiscriminately but are not interchangeable, as described in the following paragraphs (National Dairy Council, 1978; Newcomer & McGill, 1984).

Lactose deficiency or low lactase activity is defined as physiological decline of intestinal lactase activity with age (Semenza *et al*., 2001). Lactase non-persistence, often incorrectly used to refer to lactose maldigestion, is an age-related decline in lactase activity in the small intestine (National Medical Association, 2009). Lactose malabsorption is the condition where a significant amount of lactose is not absorbed, while lactose maldigestion occurs when digestion of lactose is reduced due to low activity of the enzyme lactase (Semenza *et al*., 2001; Heyman, 2006).

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Milk allergy is an abnormal response by the human immune system to milk proteins and not to lactose, and it must not be confused with lactose intolerance. It also requires complete elimination of milk and milk products from the diet (National Institutes of Health, 2009; National Institute of Allergy and Infectious Diseases, 2010). Milk allergy is primarily a childhood disease and commonly appears during infancy, with a prevalence of 2.5% in children during the first 3 years of life, while lactose intolerance occurs more often in adulthood (Businco & Bellanti, 1993; Bahna, 1996). Haenlein (2004) reported that many people who are allergic to cow milk can tolerate goat milk, because of considerable variation in goat milk protein sequences, and it is more easily digested compared with cow milk. However, goat milk proteins need more research in order to establish their non-allergic manifestation compared with cow milk.

The term "lactose intolerance" has been used to describe the various aspects of lactose metabolism. Officially, lactose intolerance is the occurrence or the development of clinical symptoms such as diarrhea, bloating, flatulence, abdominal pain, and gaseousness after intake of lactose mixed in water in a standard dose of 50 g/m^2 body surface or 2 g/kg body weight in a person with lactose malabsorption (Newcomer & McGill, 1984; Suarez *et al*., 1995). Thus lactose intolerance is the occurrence of symptoms resulting from the incomplete digestion of lactose caused by deficiency of lactase (Harrington & Mayberry, 2008; Schaafsma, 2008).

In conclusion, lactase deficiency or low lactase activity is not synonymous with lactose intolerance. Symptoms of lactose intolerance vary from individual to individual, depending on the amount of lactose consumed, and the degree of lactase deficiency. Lactose intolerance could also vary based on nutritional factors such as how the lactose is ingested (Lomer *et al*., 2008). For example, persons with milder deficiencies of lactase may be tolerant (no symptoms after ingestion of milk) to one glass of milk but intolerant (develop symptoms) to two glasses (Newcomer & McGill, 1984).

12.1.3 Symptoms of lactose intolerance

Lactose intolerance can cause a range of gut and systemic symptoms when lactose remains undigested. Food and drink that has not been labeled properly or which contains hidden lactose is hazardous for individuals with lactose intolerance. In addition to this, nutritional factors, ethnic origin and age equally affect the severity of symptoms (Cavalli-Sforza, 1973; Lloyd & Olsen, 1995; Hertzler & Savaiano, 1996). Even though lactose intolerance has been described since 400 BC, the clinical symptoms have been recognized only in the last 50 years (Matthews *et al*., 2005).

Lactose malabsorption usually occurs during early infancy and gastrointestinal symptoms appear on average after 1–2 months of feeding with cow milk (Kuitunen *et al*., 1975). Based on the quantity of ingested lactose and the person's ability to digest lactose, symptoms may vary from individual to individual. Swagerty *et al*. (2002) reported that 12–18 g of lactose are required to produce symptoms. Similarly, Suarez and Savaiano (1997) reported that ingestion of a large dose of lactose (50 g/L) can cause diarrhea, bloating, and flatulence in the majority of people with lactose malabsorption as does drinking 240 mL (8 ounces) of cow milk (Bayless *et al*., 1975). In contrast, a blind study by Scrimshaw and Murray (1988a) reported that a majority of subjects with malabsorption tolerated 240 mL of milk without noticeable symptoms. A majority of malabsorbers are still able to tolerate a certain amount of milk without any symptoms (Rosado *et al*., 1987; Scrimshaw & Murray, 1988a, b). In some cases, even after overload of lactose, the clinical picture remains asymptomatic (Suarez & Savaiano, 1994, 1997).

The common symptoms usually appear within 2 hours after ingestion of lactose and include abdominal pain, bloating, flatus, diarrhea, nausea, vomiting, and borborygmi (stomach growling) (van Griethuysen-Dilber *et al*., 1988; Gugatschka *et al*., 2005; Matthews *et al*., 2005; Suchy *et al*., 2010). These symptoms often oblige individuals to avoid dairy products in their diets. These gastrointestinal symptoms may also indicate other disorders, such as irritable colon (Suarez & Savaiano, 1994). The production of short-chain fatty acids, hydrogen, methane, and carbon dioxide during colonic fermentation of unabsorbed lactose by the bacterial microflora increases gut transit time and intracolonic pressure, which then causes abdominal pain and bloating (He *et al*., 2006). Usually, these symptoms originate from the small intestine and colon, although induction and severity of symptoms may depend on intestinal and colonic conditions such as transit time and composition of flora (van Griethuysen-Dilber *et al*., 1988).

12.1.4 Methods to quantify lactose maldigestion

Several methods have been developed to quantify lactose maldigestion or hypolactasia on the basis of records of dietary manipulation. These diagnostic tests can be carried out using direct and indirect measurements (Arola, 1994; Marteau *et al*., 1997).

12.1.4.1 Direct measurements

The first method used to study intestinal lactase activity involved jejunal biopsy (van Griethuysen-Dilber *et al*., 1988; Marteau *et al*., 1997), but the method is insensitive, invasive and provides information on lactase activity only at the site of sampling (Marteau *et al*., 1997; Shaukat

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et al., 2010). Therefore, small-bowel biopsy is rarely performed in clinical practice (Rusynyk & Still, 2001).

12.1.4.2 Indirect measurements

Indirect measurements are not invasive and the metabolism of a lactose load can be determined by changes in one of its end products in breath, urine or blood. The breath hydrogen test is commonly used, where the amount of hydrogen excreted correlates with maldigested lactose (Bond & Levitt, 1976; Arola, 1994; Marteau *et al*., 1997). This is more reliable, cost-effective, non-invasive, sensitive, and specific than the lactose tolerance test for measuring lactose maldigestion, and is positive in 90% of patients with lactose malabsorption (Shaw & Davies, 1999; Swagerty *et al*., 2002). Thus the breath hydrogen test is widely used compared with the lactose tolerance test. However, other factors such as sleep, exercise, use of aspirin, smoking, and the consumption of fermentable foods the evening before may influence the hydrogen level (Marteau *et al*., 1997; Suchy *et al*., 2010). The breath test involves the ingestion of 25–50 g of lactose and detection of carbohydrate in the colon by the measurement of excreted hydrogen and other gases (Arola & Tamm, 1994; Suchy *et al*., 2010). Before the test, lactose is given orally to fasting subjects who have less than 10 ppm hydrogen in their breath (Marteau *et al*., 1997). Undigested lactose is fermented by colonic bacteria and forms hydrogen gas. Some of the hydrogen gas is absorbed and some is excreted in the breath (McBean & Miller, 1998). Differences in hydrogen concentrations are calculated by subtracting the hydrogen concentration during fasting from the test measurements (Martini *et al*., 1991a). A breath hydrogen concentration above 20 ppm after lactose ingestion is an indication of hypolactasia (Suchy *et al*., 2010). A breath test measuring excretion of $^{13}CO_2$ after ingestion of ^{13}C-lactose and of breath radioactivity after ^{14}C-lactose ingestion can also be used but is not as common as the hydrogen breath test (Korpela, 2001).

Lactose intolerance can be determined by taking blood samples for measurement of blood glucose after ingestion of a lactose load (approximately 1–1.5 g/kg body weight) (Suchy *et al*., 2010). If the blood glucose level increases less than 20 mg/dL (1.1 mmol/L) above the fasting level, the test is considered positive (Harrington & Mayberry, 2008; Suchy *et al*., 2010). However, in 20% of normal subjects, false-positive and false-negative results may occur due to variability in gastric emptying and glucose metabolism (Suchy *et al*., 2010). The measurement of urinary galactose, with or without additional ethanol to inhibit the hepatic transformation of galactose into glucose, either quantitatively or qualitatively, using an enzymatic test strip is another simple indirect test (Marteau *et al*., 1997). Other less reliable methods include stool tests, stool pH, fecal reducing substances, and paper chromatography for the measurement of sugar in the feces. However, these methods are not recommended for research purposes (van Griethuysen-Dilber *et al*., 1988).

Even though these methods are useful for quantifying lactose maldigestion or hypolactasia, they still have several limitations. The diagnosis test can cause severe symptoms that may last for several days. Sometimes, it is difficult to distinguish between hypolactasia and lactose intolerance as there is no recommendation to monitor symptoms throughout these tests (Matthews *et al*., 2005; Harrington & Mayberry, 2008; Suchy *et al*., 2010).

12.1.5 Prevalence, age, gender, and genetics

The worldwide prevalence of lactose intolerance varies significantly among different ethnic, gender, and age groups (Johnson, 1981; van Griethuysen-Dilber *et al*., 1988; Suchy *et al*., 2010; Table 12.4). The prevalence of lactose intolerance among Europeans is less than 30% (Sahi, 1994). Primary lactose intolerance is found among 20–25% of Austrians, but Scandinavians are 95% milk tolerant, which can be attributed to the tradition of consuming dairy products by northern Europeans. However, it is still not clear what makes these populations tolerant to dairy products. It might be due to the retention of lactase activity after continued consumption of dairy products even after the weaning period (Suchy *et al*., 2010). In the worldwide population, almost 70% show lactase non-persistence but not all are lactose intolerant (Cavalli-Sforza, 1973; Hertzler & Savaiano, 1996; Matthews *et al*., 2005). In the adult world population, the lowest prevalence rate has been observed in white northern Europeans, North Americans, and Australians (Scrimshaw & Murray, 1988b; Sahi, 1994).

Table 12.4. Prevalence (%) of primary lactose deficiency in various ethnic groups.

Near East and Mediterranean: 60–100%
Asia (Thais, Indonesians, Chinese, Koreans, Japanese): 60–100%
Africa (South Nigerians, Hausa, Bantu): 60–100%
North and South America (Black Americans, Latinos, Eskimos, Canadian and American Indians, Chami Indians): 60–100%
Northern Europeans: 2–30%
Africa (Hima, Tussi, Nomadic Fulani, Masai), 2–30%
India (individuals from Punjab and New Delhi): 2–30%
Aborigines (Australia), Kiwis (New Zealand), Pacific Islanders: 2–30%

Sources: based on data from Johnson (1981), Vesa *et al*. (2000) and Schaafsma (2008).

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The prevalence is 2% in northern Europeans but almost 100% in adult Asians and American Indians (Suchy *et al*., 2010). Lomer *et al*. (2008) reported a prevalence of 5% in British populations, 17% in Finland and northern France, over 50% in South America, Africa and Asia, and in some Asian countries almost 100%. Populations of mixed ethnicity have a lower prevalence rate as compared with the native ethnic group (Johnson, 1981). Chinese and Japanese populations have a high prevalence of losing lactase activity (80–90%) within 3–4 years after weaning. Similarly, post weaning Jews and Asians lose 60–70% lactase activity over the years, while it may take up to 18–20 years for lactase activity to reach its lowest expression in white northern Europeans (Matthews *et al*., 2005). Keith *et al*. (2011) reported that 49% of African-Americans have experienced some type of physical discomfort after consumption of dairy foods, while 24% were believed to be lactose intolerant. Swagerty *et al*. (2002) reported that Blacks and Ashkenazi Jews have prevalences of 60–80%, and Latinos of 50–80%. The prevalence in the USA is 15% among whites, 53% among Mexican-Americans, and 80% in the Black population (Scrimshaw & Murray, 1988a, b; Sahi, 1994). Hypolactasia usually occurs in early childhood in Blacks and Asians, whereas in whites it is not common in childhood but occurs during adolescence (Scrimshaw & Murray, 1988b; Vesa, *et al*., 2000).

The prevalence of lactose malabsorption increases after the age of 74 years. In a cohort study of 80 healthy white women aged 40–79 years, 50% of subjects aged 60–79 were lactose malabsorbers compared with only 15% aged 40–59 (Goulding *et al*., 1999). According to Di Stefano *et al*. (2001), the prevalence of lactose malabsorption shows an increase while the prevalence of intolerance symptoms among malabsorbers shows a decrease, indicating an extended age-related decline of lactase activity during the lifespan. Therefore, lactose intolerance is one of the common disorders in an aging gut; however, the physiological mechanism for this is not clearly understood. Some of the previous studies have suggested that it is mainly due to increased enterocyte turnover in aged animals as well as in humans, indicating that relatively undifferentiated epithelial cells line the villi with functional immaturity and delayed enzyme activity (Holt *et al*., 1988, 1991; Corazza *et al*., 1998). Since there is no difference in the prevalence of hypolactasia between older and younger adults, intestinal lactase activity does not decline with age (van Griethuysen-Dilber *et al*., 1988). However, some past studies have shown the prevalence is higher in adults compared with children (Cavalli-Sforza, 1973; Welsh *et al*., 1978). Rate of prevalence based on age is still contradictory. Jussila *et al*. (1970) reported symptomatic lactose intolerance at around 46 years of age compared with those aged 31 with non-symptomatic symptoms. On the other hand, no differences were observed among the age groups 20–40 and 65 by Suarez and Savaiano (1994). Even though these results are not consistent, one possible explanation for this is the difference in hydrogen production after lactose ingestion in various age groups (Caskey *et al*., 1977; Saltzberg *et al*., 1988; Rao *et al*., 1994).

Not many studies have compared lactose intolerance between the genders. In a random population among males and females, there appears to be an equal prevalence of lactose intolerance (Rao *et al*., 1994; Rusynyk & Still, 2001). In a study by Jussila (1969), women experienced gastrointestinal symptoms and nausea after milk ingestion more often than men in a population of 504 patients. Similarly, Krause *et al*. (1996) reported a higher rate of symptoms in women compared with men despite lower hydrogen production during the test. However, during pregnancy, 45% of women can regain the ability to digest lactose (Rusynyk & Still, 2001). Even though, it seems, women are more vulnerable to gastrointestinal complaints, we cannot assume there can be differences in the amount of hydrogen excretion between the genders (Tuure & Korpela, 2004). Lactose maldigestion can also be inherited through a single autosomal recessive gene that involves low enzyme activity (Sahi & Launiala, 1977; Flatz, 1995). It has been reported that gene mutation causes a rare case of lactose intolerance in the Finnish population (Järvelä *et al*., 1998).

The lactose-intolerant population appears to vary widely among different ethnic and racial groups. Most of the reported data are based on a small group of hospital patients. Since lactose intolerance differs individually and is impacted by several physiological and psychological factors, it is difficult to discuss the true populations of lactose intolerance (McBean & Miller, 1998).

12.1.6 Non-probiotic dietary approach to alleviate lactose intolerance

Total elimination of milk and dairy products rich in nutrients from our diet could negatively impact on our health. Although dairy products containing probiotics have been used for years to alleviate lactose intolerance, lactase supplements from yeast and fungi have also been shown to reduce symptoms associated with lactose intolerance by replacing the missing lactase enzyme. There are two major sources of lactase (β-galactosidase), the yeasts *Kluyveromyces fragilis* and *Kluyveromyces lactis* or the fungi *Aspergillus niger* and *Aspergillus oryzae* (Solomons *et al*., 1985b; Rao, 1997), and these have found industrial application for many years. Production of β-galactosidases from various strains of *A. niger* and *A. oryzae* are used commercially for the hydrolysis of lactose in whey, for the alleviation of

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Table 12.5. Commercial over-the-counter lactase supplements.

Source	Product	Supplier
Aspergillus niger	Dairy Ease chewable tablets	Glenbrook Laboratories, New York, NY, USA
	Lactrase capsules	Schwarz Pharmaceuticals, Milwaukee, WI, USA
Aspergillus oryzae	Lactogest soft gel capsules	Thompson Medical Co., Inc., New York, NY, USA
Aspergillus niger and *Aspergillus oryzae*	Dairy digest complete	NOW Foods, Bloomingdale, IL, USA
Bacillus coagulans GBI-30, 6086	Digestive advantage	Schiff Nutrition International, Salt Lake City, UT, USA
Kluyveromyces lactis	Lactaid caplets	Lactaid, Inc., Pleasantville, NJ, USA

Source: based on data from Rao (1997).

lactose intolerance, and for the production of galacto-oligosaccharides (Nakayama & Amachi, 1999). In US markets, several over-the-counter lactase supplements (Table 12.5) prepared from these sources are available, including milk supplemented with lactase called "Lactaid" (Rao, 1997; O'Connell & Walsh, 2008).

Pharmaceutical preparations of fungal- or yeast-derived β-galactosidase have been shown to increase lactose digestion and alleviate symptoms (Solomons *et al*., 1985b; Moskovitz *et al*., 1987; Sanders *et al*., 1992). Other than complete elimination of lactose-containing foods, these oral supplementations are major treatments for alleviating symptoms of lactose intolerance (Gaska, 1990). The enzymes used in these digestive supplements are obtained from "Generally Recognized as Safe" (GRAS) listed fungi such as *A. niger* and *A. oryzae* (Gaska, 1990; O'Connell, 2006; O'Connell & Walsh, 2008). Lactase obtained from *A. oryzae* has been considered more desirable due to its higher residual activity at the natural pH (6.7) of milk (Bailey & Linko, 1990; Tuure & Korpela, 2004). These products are less effective compared with the lactose fermented in yogurt or in pre-hydrolyzed milk (Onwulata *et al*., 1989; Shaw & Davies, 1999). Previous studies on lactase-based supplements have reported limited effects in alleviating the symptoms of lactose malabsorption (O'Connell & Walsh, 2006a). Similarly, Ramirez *et al*. (1994) and Moskovitz *et al*. (1987) mention that these supplements vary in their effectiveness and that they do not work for all lactose-intolerant people. Binkley (1996) has also reported an allergic reaction with the ingestion of *Aspergillus*-derived lactase supplement. Even though some of the controlled clinical trials have shown the effectiveness of these supplements, the results have been variable and not consistent (Rao, 1997). O'Connell and Walsh (2006b) suggested that none of the currently available commercial products meet the typical characteristics of an ideal supplemental lactase and a higher dosage of these supplements is required to completely hydrolyze the lactose found in dairy products. However, purified enzymes from *Aspergillus* and yeast displayed significant stability when exposed to simulated gastric conditions and may be suitable for use as a digestive supplement for the alleviation of lactose intolerance (Nakayama & Amachi, 1999). Rosado *et al*. (1984) assessed the efficacy of two enzymes produced from yeast and fungus in adult lactose malabsorbers. Their study showed that the addition of enzyme directly to milk during their mealtime 5 min before consumption significantly reduced the symptoms of lactose intolerance. Even though the role of β-galactosidases derived from yeast (*Kluyveromyces)* in reducing lactose intolerance has been explained in previous research, their stability and potential application under simulated intestinal conditions as a digestive supplement has not been well discussed. These supplemental lactases are active during the initial stages of gastric digestion but perform significantly less well in the small intestine due to the acidic condition. O'Connell and Walsh (2006b) also reported that purified neutral lactase from *Kluyveromyces marxianus* is significantly more active in the small intestine than current commercial products. Although purified enzymes obtained from *Aspergillus* have been widely used for industrial applications, the purification method is considered to be a challenge and can be costly. Therefore, the better alternative is the use of probiotic bacteria as a source of these enzymes (Alazzeh *et al*., 2011).

12.1.7 Intestinal microflora, fermentation, and fermented foods

Fermented milk products have been shown to improve lactose digestion and lactose intolerance (Montalto *et al*., 2006). He *et al*. (2008) reported that supplementation of yogurt or probiotics modified the composition and metabolism of the colonic microbiota in healthy adults, healthy infants and patients with functional bowel disorders. Montalto *et al*. (2006) has also mentioned that commercially

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available yogurt is effective in reducing the symptoms of lactose intolerance due to the presence of endogenous lactase activity of mainly two species of lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). According to McDonough *et al*. (1987) and Gorbach (1990), fermentation reduces lactose content by approximately 25–50%. It has been shown that yogurt delays gastric emptying and intestinal transit, causing slower delivery of lactose to the intestine and improving the activity of β-galactosidase in the small bowel, which helps to decrease the osmotic load of lactose (de Vrese *et al*., 2001; Labayen *et al*., 2001). Therefore, the symptoms of lactose intolerance are influenced by the amount of lactose ingested, small intestinal lactase activity, and colonic processing of lactose (van Griethuysen-Dilber *et al*., 1988; Vesa *et al*., 2000).

Alteration of the composition and metabolism of the colonic microbiota may be achieved through the use of dietary supplementation (probiotics, prebiotics and synbiotics) (Collins & Gibson, 1999). Previous studies have shown that lactose digestion and the symptoms of lactose intolerance can be improved using probiotics by modifying gut pH, expressing β-galactosidase, and exerting positive effects on intestinal activity and overall colonic microbiota (Roberfroid, 2000; Rolfe, 2000; de Vrese *et al*., 2001; Kopp-Hoolihan, 2001). On the other hand, Levri *et al*. (2005) reported that probiotic supplementation did not improve the condition of lactose intolerance in adult populations.

The human gastrointestinal tract contains approximately 17 bacterial families comprising over 500 species, with the colon containing 10^{12} to 10^{14} organisms/mL (Lomer *et al*., 2008). It has been demonstrated that lactic acid bacteria ferment lactose to lactate, hydrogen, methane, carbon dioxide, and short-chain fatty acids (Hove *et al*., 1999). During fermentation lactase present in lactic acid bacteria cleaves unabsorbed lactose to glucose and galactose and these are absorbed. In the small intestine at pH 6–8, lactase activity is optimal; however, in the colon pH decreases to 4 and lactose is left unfermented due to the decreased bacterial lactase activity (Heyman, 2000). Undigested lactose can be considered to be a prebiotic that stimulates the growth of beneficial microflora by fermenting milk containing less lactose. The increase in number of lactic acid bacteria is due to microbial digestion during fermentation (Lin *et al*., 1993).

The level of lactose intolerance in different individuals is due to the variable ability of colonic microflora to ferment lactose (Arola & Tamm, 1994). Dairy products such as cheese and fermented milk (yogurt) do not lead to the symptoms of lactose intolerance. Besides consuming live culture yogurts, there are several effective ways to increase dairy food consumption. For instance, a variety of lactose-free dairy products such as Lactaid milk, yogurt, cheese and ice cream or other Lactaid supplements are available (Heaney *et al*., 2011). Lactase milk contains lactase, which is not present in regular milk and its products. This natural enzyme breaks down lactose into simple sugars that are easily digestible and individuals can enjoy the benefit of milk without experiencing the symptoms of lactose intolerance. Consumption of Lactaid milk produces a significant decrease in the excretion of hydrogen during breath-analysis tests for lactose maldigestion (Solomons *et al*., 1985a).

12.1.8 Use of probiotics to alleviate lactose intolerance

Probiotics are live microorganisms that when ingested produce beneficial effects in the prevention or treatment of diseases (McFarland & Elmer, 1995). Probiotics contain β-galactosidase (lactase) and the consumption of probiotics can be beneficial in various human illnesses including lactose intolerance. The most commonly used probiotics are lactobacilli present in yogurt (Ouwehand *et al*., 2002). The alleviation of lactose intolerance by the use of probiotics is a widely accepted health claim. Other microbes and yeasts have also been used as probiotics (Table 12.6).

Increased numbers of yogurt bacteria (from 7 log to 8 log CFU/mL) have improved lactose digestion. Gallagher *et al*. (1974) reported that consumption of fermented dairy products (buttermilk, yogurt and cottage cheese) were well tolerated by lactase-deficient people whereas non-fermented dairy products (milk, ice cream and milk powder) caused symptoms of intolerance. Lactose ingested with yogurt produces low amounts of hydrogen excretion compared with ingestion of the same amount of lactose in water or milk (Kolars *et al*., 1984). Better lactose digestion has been observed after ingestion of yogurt containing live bacteria than after ingestion of heated yogurt (Gilliland & Kim, 1984). A lower level of breath hydrogen as well as unchanged β-galactosidase activity was also observed in subjects who consumed yogurt containing live bacteria compared with those who consumed pasteurized yogurt (Savaiano *et al*., 1984; Pochart *et al*., 1989). Higher levels of enzyme activity in feces indicate significant amounts of lactose in the colon after ingestion of heated yogurt. It has also been reported that the effect of yogurt is the same regardless of the strain of bacteria. Improvement in lactose digestion has been shown when *Lactobacillus acidophilus* cells were sonicated (McDonough *et al*., 1987). Yogurt bacteria containing high levels of lactase are rapidly released when the bacteria are lysed by bile salts in the gastrointestinal tract (Marteau *et al*., 1990). However, *L. acidophilus* can also be active but resistance to bile makes it less efficient than other yogurt bacteria (Marteau *et al*.,

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Table 12.6. Effectiveness of probiotics for the treatment of lactose intolerance.

Experimental treatments	Associated actions	Reference
Non-fermented milk with yogurt culture	Improved lactose digestion	Lin *et al*. (1991)
Fermented dairy products (buttermilk, yogurt, cottage cheese)	Well tolerated by lactose intolerant	Gallagher *et al*. (1974)
Ingestion of lactose in milk, water or in yogurt	Lactose in yogurt resulted in about one-third of the H_2 breath excretion compared with water or milk	Kolars *et al*. (1984)
Ingestion of yogurt with live bacteria vs. ingestion of heated yogurt	Better lactose digestion after ingestion of live bacteria	Gilliland & Kim (1984); Savaiano *et al*. (1984)
	Fecal β-galactosidase activity increased in lactase-deficient subjects who ingested heated yogurt, indicating significant amount of lactose in colon	Pochart *et al*. (1989)
Yogurt consumed with or without meal	Reduced lactose maldigestion	Martini *et al*. (1991a)
Yogurts made with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* consumed with or without a meal	Improvement in lactose maldigestion was similar regardless of strains	Martini *et al*. (1991b)
Milk with sonicated *Lactobacillus acidophilus*	Improvement in lactose digestion	McDonough *et al*. (1987)
Fermented semi-solid milk containing *L. acidophilus*, *Bifidobacterium* sp. and yogurt bacteria	No differences in the digestibility	Marteau *et al*. (1997)
Feeding cultured buttermilk with *Lactococcus lactis* subsp. *lactis*	Did not improve lactose digestion	Savaiano *et al*. (1984)
Ingestion of lactase isolated from yeasts and molds in the form of capsules	Increases lactose digestion	Corazza *et al*. (1992)
S. thermophilus produces β-galactosidase during its transit in the digestive tract of mice	Reduces the lactose content in digestive tract	Drouault *et al*. (2002)
Lactobacillus strains	Lactose digestion improved, decreased diarrhea and symptoms of lactose intolerance	Marteau *et al*. (2001)
Lactobacillus plantarum	Reduced bloating, flatulence, and pain in irritable bowel	Nobaek *et al*. (2000)
Saccharomyces boulardii (yeast)	Decreased only functional diarrhea, but not any other symptoms of irritable bowel syndrome	Marteau *et al*. (2001)
Milk containing *L. acidophilus*	No more effective than regular milk in reducing gastrointestinal symptoms in the study of self-reported lactose-intolerant individuals	Semenza *et al*. (2001)

1997). There were no differences in digestibility between fermented milks containing *L. acidophilus*, *Bifidobacterium* sp. or other yogurt bacteria. Savaiano *et al*. (1984) did not find improvement in lactose digestion when using cultured buttermilk with *L. lactis* containing only a phosphor-β-galactosidase. Corazza *et al*. (1992) found an increase in lactose digestion when lactase from yeasts and molds was taken in the form of capsules. *Streptococcus thermophilus*

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produces a β-galactosidase during transit of the digestive tract of mice, and this enzyme reduced the lactose content by hydrolyzing it, resulting in an overall reduction of lactose in the feces (Drouault *et al*., 2002). Administration of other probiotic *Lactobacillus* strains such as *Lactobacillus plantarum* has been shown to improve lactose digestion and decrease diarrhea and reduce bloating, flatulence and pain in individuals with irritable bowel (Nobaek *et al*., 2000; Marteau *et al*., 2001). Ingestion of *Saccharomyces boulardii* has not improved any symptoms of irritable bowel but has been shown to reduce functional diarrhea (Marteau *et al*., 2001). In a clinical intervention study of lactose intolerance, Shaukat *et al*. (2010) did not observe any effect of milk containing *L. acidophilus* compared with regular milk in reducing gastrointestinal symptoms.

The combination of probiotic supplement and non-fermented dairy product does not reduce the symptoms of lactose intolerance in adults. However, some specific probiotic strains, concentrations, and methods of preparation might be effective (Martini *et al*., 1991b). Therefore, some probiotics only improve lactose digestion but do not alleviate the symptoms of lactose intolerance. Intake of yogurt causes fewer symptoms than intake of milk mainly due to the high β-galactosidase activity in yogurt, partial hydrolysis of lactose, and slower intestinal transit because of the digestion of lactose in yogurt (Cox, 2003; Rabot *et al*., 2010). He *et al*. (2008) reported a decrease in lactose content by approximately 20–40% during fermentation by lactic acid bacteria. Their study showed that supplementation with *Bifidobacterium longum* (in capsules) and a yogurt enriched with *Bifidobacterium animalis* altered the amount of bacteria and increased the β-galactosidase activity in feces from lactose-intolerant persons. Lactic acid bacteria in fermented milk increase lactase activity in the small intestine and thus provide beneficial effects (Gilliland & Kim, 1984; Fernandes *et al*., 1987). However, it is still not clear whether yogurt is supplying the lactase or whether the bacteria produce lactase when they enter the gut (Fuller, 1991). Lin *et al*. (1998) found that *L. bulgaricus* is a better choice for manufacturing non-fermented milk products than *L. acidophilus* because their cell wall membrane structure is less tough than that of *L. acidophilus*. *Streptococcus thermophilus* strains have been shown to contain more lactase than strains of lactobacilli or bifidobacteria. Hence, the consumption of ordinary yogurt or milk fermented by *S. thermophilus* is the best way to improve lactose intolerance (Kolars *et al*., 1984; Sanders *et al*., 1996).

Consumption of fermented dairy products containing specific probiotic strains in appropriate amounts should be incorporated into the diets of lactose-intolerant subjects. The effectiveness of probiotics also depends on the tolerance of a strain to bile and acid besides its lactase level or lactose transport (Mustapha *et al*., 1997). Several studies have been carried out to find alternative approaches for enhancing β-galactosidase activity. For this, selection of probiotic strains capable of producing large amounts of β-galactosidase is important. Selection of strains that help to improve lactose digestion should exhibit resistance to the conditions encountered in the digestive system (Rodriguez *et al*., 2003). The most extensively studied and widely used probiotics are the lactic acid bacteria, particularly *Lactobacillus* and *Bifidobacterium* spp. This food-grade classical approach has the ability to moderately increase β-galactosidase concentrations in probiotic cultures to improve their potential for treating the symptoms of lactose malabsorption in humans.

Ibrahim and O'Sullivan (2000) have developed a classical chemical mutagenesis protocol to increase β-galactosidase production by probiotic bacteria to improve their potential to treat symptoms of lactose malabsorption in humans, and which showed great promise for assessment of probiotic functionality. Two *Bifidobacterium* species (*B. breve* and *B. longum*) and one strain each of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were tested by single exposure to two chemical mutagens, ethyl methanesulfonate and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Mutants showed increased β-galactosidase activities compared with the wild-type strains. Similarly, Alazzeh *et al*. (2011) reported that chemical mutagenesis of *Lactobacillus reuteri* led to enhancement of β-galatosidase, indicating an effective method for enhancing enzymatic activity.

Previous studies have demonstrated the production of β-galactosidase from non-food-grade microorganisms (Ibrahim & O'Sullivan, 2000; Hsu *et al*., 2005; Donkor *et al*., 2007) and which are not approved for food use. Alazzeh *et al*. (2009) have shown the influence of different carbohydrate and protein sources in enhancing the production of α- and β-galactosidases in six strains of *L. reuteri*. Based on their study, raffinose and lactose were the best carbohydrate sources for producing α- and β-galactosidases, respectively. Yeast extract was the best protein source for producing both enzymes, and *L. reuteri* strain CF2-7F was the best-producing strain under all experimental conditions. Therefore, these selected strains could be added as a food-grade additive to various foods and could provide benefits mainly to lactose-intolerant individuals. The production of α- and β-galactosidases from *L. reuteri* with addition of different metal ions has been studied. Ibrahim *et al*. (2010) reported the enhancement of α- and β-galactosidase activity when Mn^{2+} ions were added to *L. reuteri* strain CF2-7F. Chowdhury *et al*. (2007) reported

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higher production of β-galactosidase from yogurt containing *L. acidophilus* and *L. plantarum* fortified with different types of herbs. Increase in β-galactosidase activity was attributed to the antioxidants present in the herbs. Similarly, Bhowmik *et al*. (1987) evaluated the influence of different growth conditions on the activity of β-galactosidase in *L. acidophilus* and found that enzyme activity was stimulated by magnesium.

12.2 CONCLUSIONS

Lactose intolerance has a global prevalence and is one of the most common gastrointestinal disorders. Lactose-intolerant persons usually avoid milk and dairy products in order to improve gastrointestinal disorders. Frequently, lactose intolerance is confused with other gastrointestinal disorders such as diarrhea and abdominal bloating and is thus misdiagnosed. However, the lactose-intolerant person can be well treated by dietary modification and education once the condition is diagnosed properly.

Milk must be consumed in amounts that can be tolerated by the lactose-intolerant individual rather than being completely eliminated. Avoidance of milk and dairy products can lead to nutritional deficiency, particularly of calcium (Lomer *et al*., 2008). In addition, lactose is an excellent source of energy; however, for effective utilization, lactose needs to be hydrolyzed in the intestine by β-galactosidase, generally called lactase (Lomer *et al*., 2008). Lactose maldigesters can include milk and other dairy products in their diet without experiencing symptoms with some modification in their dietary pattern. It is well known that lactose-intolerant people can replace milk with yogurt or other fermented dairy products (Adolfsson *et al*., 2004). Probiotics including yogurt bacteria that contain high levels of lactase have been shown to alleviate lactose intolerance (Marteau *et al*., 1997). Therefore, future studies should focus on selection of the probiotic strains that can enhance the production of β-galactosidase. Hence, the addition of such probiotics in various food products seems to be the most effective means of alleviating lactose intolerance. As there are no reliable and simple methods for diagnosis of lactose intolerance that would be effective for all clinical symptoms, thorough studies should be conducted globally to determine the prevalence of lactose intolerance among different age, racial and ethnic groups.

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13
Milk Quality Standards and Controls

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13.1 INTRODUCTION

Production, processing, and distribution of high-quality milk and its product are important steps for food safety, human health, and nutrition. Milk and dairy products must be safe to consume and be free of pathogenic bacteria and antibiotic, insecticide and herbicide compounds. They should have good and no objectionable flavors, be free of spoilage bacteria, and contain legal minimum limits of all nutrients (Loewenstein *et al*., 1984; Park & Guo, 2006).

All standards and regulations pertaining to production, processing, and marketing of milk and products in the USA are described in a publication of the federal government (Food and Drug Administration, FDA) called the Grade A Pasteurized Milk Ordinance (US PMO, 2009) (Peters, 1990; Park & Guo, 2006). It has evolved from earlier publications from the US Surgeon General (US Public Health Service, 1939) and contains great detail about the requirements for sanitary facilities, equipment and practices for the production of safe, quality cow milk. It also spells out definitions of nutritional standards, including inspection requirements and score cards, and states in section 1-I that "Goat milk is the lacteal secretion, free from colostrum, obtained by the complete milking of healthy goats, and shall comply with all the requirements of this ordinance. The word 'cows' shall be interpreted to include goats." In the European Union (EU), similar standards are enforced such as Codex or International Dairy Federation (IDF) regulations.

Milk quality may be evaluated by parameters assessing the suitability of raw milk for consumption, the processing of the milk for dairy products, and the health status of individual animals producing the milk. Thus milk quality may be evaluated through measurement of total bacterial count or the presence of specific types of bacteria, and somatic cell count (SCC) or numbers of leukocytes per milliliter of milk. Differential SCC using flow cytometry can be used for the evaluation of udder health and leukocyte cell types in milk (Albenzio & Caroprese, 2011). Recently, Albenzio *et al*. (2012) reported that the association between somatic cells, cytokines, endogenous proteolytic enzymes, and pathogenic bacteria can be used to better understand the pathogenesis of subclinical mastitis in ewes and the effect on the immune response of the ewe mammary gland. Numerous studies, mostly on cow milk, have demonstrated that an increase in SCC causes a decrease in milk yield and affects milk composition, which results in reduced suitability for cheesemaking (Politis & Ng Kwai Hang, 1988; Barbano *et al*., 1991). However, little is known about how SCC affects this complex process in goat and sheep milk, which is mainly used for cheese manufacture.

Many factors have been reported to influence milk composition and yield, which are directly related to the quality of milk and its products. Diet, breed, parity, stage of lactation, type of birth, disease, estrus, diurnal, monthly and seasonal variations, and environmental temperature

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(Schmidt, 1971; Gonzalo *et al*., 2002, 2005; Haenlein, 2002; Park & Guo, 2006) significantly contribute to changes in SCC in goat and sheep milk. These factors may explain 48% of SCC variance (Gonzalo *et al*., 2002).

The purpose of this chapter is to review and address the key issues involved in milk quality standards and quality control, production and processing of quality milk and its products in relation to human consumption, well-being and nutrition.

13.2 GENERAL PRINCIPLES FOR PRODUCTION OF QUALITY MILK

Production of high-quality raw milk is of paramount importance for successful manufacture and marketing of dairy products. Production of high-quality milk for any dairy species largely depends on the farm producer as well as workers at dairy processing plants, dairy distributors and retail stores. Quality milk production must start at the farm level, because the flavor and quality of the milk cannot be improved later in the processing stages (Park & Guo, 2006; Park, 2010). The general principle of quality dairy production is that the better the raw milk, the better the processed products.

The natural raw milk produced from the mammary glands is highly perishable, and its quality is easily influenced negatively by many factors such as feeding, handling of animals prior to and during milking, handling of the milk during and after milking, cooling, transportation, pasteurization, processing, packaging, and processing utensils (Peters, 1990; Haenlein, 1993). A clean environment in the milking parlor and milking barn is just as important as the composition of the raw milk. Because milk is a highly nutritious medium for bacterial growth, it is liable to deteriorate rapidly. Good-quality milk must contain no harmful pathogens or microorganisms likely to damage the cultured dairy products, nor such foreign substances as antibiotics, antiseptics or pesticide residues (Kosikowski, 1977; Loennerdal *et al*., 1981; Le Jaouen, 1987). In addition, the basic bacterial flora should not be too numerous.

On commercial milk production farms and processing plants, at least five major parameters are routinely monitored by various regulatory and government agencies in order to safeguard quality milk production. These parameters include:

1. the nutritional constituents in milk;
2. SCCs as related to mastitis;
3. bacterial counts as related to sanitary practices;
4. the adulteration and pesticide residue contents; and
5. the flavor, taste, appearance, and temperature (Haenlein, 1993; Park & Guo, 2006; Park, 2010).

Fresh milk can have off-flavors. Off-flavors in raw milk can be attributed to feed, weeds, forages, chemicals, building materials, colostrum, estrus, mastitic milk, filthy utensils and strainer, unclean milking equipment, slow cooling, odors from bucks, barn and/or milk room. Feeding odorous feeds such as pasture containing garlic less than 2 hours before milking is not recommended (Park & Guo, 2006). Short-chain free fatty acids (butyric, capric, caproic and caprylic acids), when generated by lipase in goat milk due to improper milking procedures and cooling, are considered to cause goaty and rancid tastes (Park & Guo, 2006).

Good-quality milk can be produced only through good management of the entire farm system. This should include following the recommended milking practices in a daily routine, by maintaining functioning and sanitary equipment, by having healthy animals, and by using recommended detergents, acids and sanitizers for cleaning and milking equipment (Park, 2010).

13.3 REGULATORY STANDARDS OF QUALITY MILK AND DAIRY PRODUCTS FOR DIFFERENT SPECIES

There are differences in milk secretion processes between dairy species. Goats secrete milk through the apocrine process, whereas cows produce milk by the merocrine process. Although goat milk contains naturally higher SCCs than cow milk due to the apocrine secretory process, the SCC regulations for milk quality standards are enforced for both species. Goat milk commonly has a high SCC even when the actual numbers of leukocytes in its milk are relatively low (Kapture, 1980; Park & Humphrey, 1986; Haenlein, 1996; Park, 2010). Because of this discrepancy, dairy goat producers on the National Conference of Interstate Milk Shipments have very actively pursued this problem of SCC legal thresholds (Kapture, 1980; Haenlein, 1993; Haenlein & Hinckley, 1995).

SCC has been used as a quantitative index for mastitic conditions or degree of glandular irritation in the mammary gland (Poutrel & Lerondelle, 1983; Park & Humphrey, 1986; Park & Guo, 2006). Generally, milk with high somatic cells and spoilage bacteria results in poor-quality products. SCC can be determined by various cowside and electronic tests including the California Mastitis Test and Wisconsin Mastitis Test. The standard rules and regulations for all aspects of production, processing, and marketing milk in the USA are described in US PMO (2009). From these standards for Grade A milk, each state health department establishes its minimum regulations (Peters, 1990; Park & Guo, 2006). In the EU, similar standards are enforced through Codex or IDF regulations (International Dairy Federation, 1995). Some states in the USA may adopt more stringent standards than the PMO regulations. The state of Oregon, for example, has set its SCC standard at 750 000 cells/mL, while the PMO standard is 1 000 000 cells/mL. In the state of Georgia, recently the SCC for goat milk has been raised to 1 000 000 cells/mL as the maximum cell count (Park & Guo, 2006; Park, 2010).

SCC measures the number of different types of cells in milk, mainly represented by lymphocytes, macrophages,

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polymorphonuclear leukocytes (PMNs), and epithelial cells. Health programs for dairy species have been significantly enhanced in the last decades and several laws have been issued worldwide. In the EU, Regulation 853/2004, laying down specific hygiene rules for food and animal origin (European Union, 2004), states that raw cow milk must have an SCC lower than or equal to 400 000 cells/mL, and a total microbial count (TMC) lower than or equal to 100 000/mL. For raw milk from other species, the only criteria specified is a TMC value lower than or equal to 1 500 000/mL; for raw milk from other species where the manufacturing process does not involve any heat treatment, a TMC value lower than 500 000/mL is specified. Bulk SCC is also sometimes regarded as an index of herd welfare; Scandinavian countries tend to regard herds with bulk total SCC above 400 000 cells/mL as having poor welfare and being unhealthy overall. In the EU there is no legal limit for SCC in goat and sheep milk. The systematic extrapolation of findings from investigations on cows to small ruminants leads to errors in the diagnosis of subclinical intramammary infection (IMI) and in the application of discriminatory standards for sheep and goat milk quality (Raynal-Ljutovac *et al*., 2007). Sevi *et al*. (1999) suggested a threshold of 700 000 cells/mL for ewe bulk milk of satisfactory hygienic and processing quality. Albenzio *et al*. (2011) found impairment of mammary epithelium secretory efficiency starting at an SCC of 300 000 cells/mL in ewe milk. Rosati *et al*. (2005) proposed a cut-off value of 265 000 cells/mL for SCC in ewe bulk milk to discriminate between infected and non-infected ewe udders. The Scientific Panel on Biological Hazards of the European Food Safety Authority (EFSA) states that high SCC cannot be used as a marker of udder infection in goat milk (European Food Safety Authority, 2005).

There are at least four important requirements for Grade A milk with regard to its quality: (i) safe to drink, (ii) good flavor, (iii) relatively free from spoilage bacteria and somatic or body cells, and (iv) composition (Loewenstein *et al*., 1984; Park, 2010). Pasteurization is the most important method for killing pathogens to ensure safe milk, but it does not remove other contaminants. It must be stressed that raw milk has the best flavor when it comes from clean, healthy, properly managed dairy herds, including goats, and that the ideal flavor is slightly sweet and slightly salty with a complete absence of strong odors and flavors. An oxidized flavor is attributed to churning of milk in high pipelines in milking parlors, elbows in pipelines, nutritional imbalances of the dairy animals, or exposure of bottled milk to light. All milk, especially goat milk, can develop rancid, off or goaty flavors when the milk fat is partially disintegrated by enzyme action. These off-flavors can be controlled by proper rapid cooling after milking, by pasteurization and by protection of the milk from sun and ultraviolet light (Park & Guo, 2006; Park, 2010).

All Grade A pasteurized milk and milk products must be produced, processed, and pasteurized to conform with the specific PMO codes. The "standard" milk composition for the fluid milk market refers to the levels of major nutrients such as fat, protein, lactose, and minerals. A US Public Health Service agency, the FDA, defines milk as containing a minimum of 3.25% fat and 8.25% milk solids not-fat, which is the sum of protein, lactose, and mineral contents. The FDA standards are enforced for cow milk, while the same definition and regulations have been applied to goat milk though differences in composition exist between the two species (Park & Guo, 2006; Park, 2010).

With regard to quality standards for cow milk, an example of the chemical, bacteriological, and temperature standards, and the sanitation requirements is shown in Table 13.1 (Colorado Department of Health, 1980). In addition, the quality control guidelines for microbiological standards in dairy foods are presented in Table 13.2 (Harper, 1980). These regulations and guidelines may be applied to milk from other species such as goat and sheep. There are many laboratory testing procedures to control contamination with water, dirt, antibiotics, off-flavors, and thermoduric, psychrotrophic and coliform bacteria. These milk quality parameters include, but are not limited to, physicochemical and enzymatic indices as well as specific milk components, including specific gravity, freezing point, SH value, titratable acidity, redox potential, electrical conductivity, and enzyme levels (i.e., alkaline phosphatases, lipoprotein lipases and proteinases) (Park, 2010).

In addition to visual inspection by the milk tanker driver on loading milk from farms, daily laboratory tests are performed for nutritional content and whether the milk delivered at the processing plant meets physical and bacterial requirements (Haenlein, 2001). Each truckload is sampled and tested for seven criteria before it can be unloaded:

1. visual color and physical condition (normal);
2. flavor (absence of off-flavors);
3. temperature (< 5°C);
4. dirt and sediment score (low);
5. a cryoscopic check on water contamination (−0.530 to −0.550°C);
6. free of antibiotics; and
7. SCC (< 1 000 000 cells/mL for goat milk, < 400 000 cells/mL for cow milk).

A state inspector and a veterinarian visit dairy farms at least once a year to review facilities, the milking conditions, using a detailed 100 point-score card, and the health of the dairy animals and their udders. A federal inspector may also visit the farm each year. In addition, most milk processing companies provide incentive payments to producers for meeting or exceeding minimum nutrient contents and bacterial standards, or apply deductions to

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Table 13.1. Chemical, bateriological, and temperature standards.

Grade A raw milk for pasteurization
Temperature: cooled to 7°C (45°F) or less within 2 hours after milking, provided that the blend temperature after the first and subsequent milkings does not exceed 10°C (50°F)
Bacterial limits: individual producer milk not to exceed 100 000/mL prior to comingling with other producer milk. Not to exceed 300 000/mL as comingled milk prior to pasteurization
Antibiotics
Individual producer milk: no detectable zone with the *Bacillus subtilis* method or equivalent
Comingled milk: no detectable zone by the *Sarcina lutea* cylinder plate method or equivalent
Somatic cell count: individual producer milk not to exceed 1 500 000/mL

Grade A pasteurized milk and milk products
Temperature: cooled to 7 °C (45 °F) or less and maintained thereat
Bacterial limits: 20 000/mL*
Coliform: not to exceed 10/mL, provided that, in the case of bulk milk transport tank shipments, shall not exceed 100/mL
Phosphatase: less than 1 μg/mL by the Scharer rapid method or equivalent
Antibiotics: no detectable zone by the *Sarcina lutea* cylinder plate method or equivalent

*Not applicable to cultured products.
Sources: based on data from Colorado Department of Health (1980). Also cited in Park & Guo (2006) and Park (2010).

Table 13.2. Quality control guidelines for microbiological standards in dairy foods.

Product	Standard plate count	Coliform	Psychrotrophic (SPC after 5 days at 7°C)	Yeast and molds	Staphylococci	*Salmonella*
Raw milk	<1000–50 000	<100–<1000	<10 000–<100 000	—	—	—
Bulk tankers						
Comingled raw milk at pasteurizer	<50 000–30 000	<100–<1000	100 000–<800 000	—	<5000–<100 000	—
Pasteurized Grade A fluid products	<1000–<10 000	<1–<5	<20 000–<69 000	—	<1	<1
Ice cream	<20 000–50 000	<1–<10	<50		<1	<1
Cottage cheese (dry)	<1000–20 000	<1–<5	<10 000–<100 000	<5–<10	<1	<1
Butter	<5000–<20 000		<50 000	<5–<10	<1	<1
Milk powder	<20 000–<50 000	NS	NS	<10	<1	<1

Source: based on data from Harper (1980).

payment for failing to meet these requirements (Table 13.3) (Haenlein, 2001).

13.4 QUALITY CONTROL PRINCIPLES FOR MILK PRODUCTION ON DAIRY FARMS

Animal health management and product quality control have evolved from a solely curative veterinary practice to the application of veterinary herd health and production management programs, where herd performance is monitored in an operational setting in order to better prevent disorders. Recently, more emphasis has been placed on the issue of milk quality and the production process. There is an increasing tendency for livestock production to be integrated into the food production chain, from farm level up to consumer level. Hazard Analysis Critical Control Point (HACCP) is a systematic approach to identification, assessment and control of hazards, developed in the late 1960s to ensure the safety of foods for space flights. It was subsequently recognized as an effective alternative to conventional end-point testing by the World Health

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Table 13.3. Example of a milk quality incentive program used by one milk processing company in Wisconsin, USA, based on their laboratory tests for monthly payments to cow milk producers.*

PLC (1000) Standard plate bacterial count	PI (1000) Preliminary incubation bacterial count	SCC (1000) Somatic cell count	Cryoscope degrees below 0°C freezing	Payment deduction (–) or bonus (+) in $/45 kg milk
>100	>200	>750	<0.530	–0.10
50–100	100–200	600–750	<0.530	–0.05
<20	<40	<300	0.530–0.550	+0.10
<10	<30	<225	0.530–0.550	+0.20
<5	<20	<150	0.530–0.550	+0.40
<3	<10	<125	0.530–0.550	+0.50

*Antibiotics must always be negative, farm inspection score must always be >90 points.
Courtesy of Dr G. Haenlein.

Organization (WHO) and the United Nations Food and Agriculture Organization (FAO), among others, and recommended for use in commercial food production. HACCP principles are now incorporated in the national food safety legislation of many countries, as well as being a likely future component of the standardization of international food quality control and assurance practices. The HACCP concept has been proposed as an alternative quality control system for dairy farms, because it addresses hazards and risks and focuses on preventive risk management in a farm-specific setting. A quality assurance program starting at dairy farm level deals with animal health, animal welfare and food safety issues to take account of the demands of consumers and retailers during daily work, or in the framework of heard health. The HACCP concept appears to be very promising for application on farms because it is farm-specific, relatively low in labor and record-keeping demands, focused on risk management and prevention, easy to link to both operational management and food chain quality assurance, and suitable for certification.

13.5 HACCP PLANS AND HAZARD COMPONENTS IN THE PRODUCTION OF QUALITY DAIRY PRODUCTS

In order to produce quality Grade A milk and its manufactured products, it is recommended that HACCP plans be implemented in order to establish better control systems at dairy processing plants. Seven well-known principles need to be implemented:

1. conduct a hazard analysis;
2. identify critical control points;
3. establish critical limits for each critical control point;
4. establish monitoring procedures;
5. establish corrective actions;
6. establish record-keeping procedures; and
7. establish verification procedures (Park & Guo, 2006; Park, 2010).

Modifications to the HACCP plan can be made for milk and different manufactured products at various locations depending on the specific situation of individual processing plants.

The hazards associated with the production of milk and dairy products are classified as microbiological, chemical, and physical hazards. The Center for Food Safety and Applied Nutrition of the US FDA has delineated these three types of hazards, potential hazards, and hazard management or controls (extensively illustrated in Tables 13.4 and 13.5). Table 13.4 specifically describes the hazards and controls for milk plant raw materials, while Table 13.5 shows the partial list of hazards and controls of milk plant processing operations.

The posssible microbiological hazards involved are as follows:

1. Raw milk: *Salmonella*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Brucella*, *Campylobacter*, *Clostridium perfringens*, *Listeria monocytogenes*, *Shigella*, *Yersinia*, and staphylococcal enterotoxin.
2. Cheese: *L. monocytogenes*, *Campylobacter*, *Shigella*, *Brucella*, *Salmonella*, *Clostridium botulinum*, *S. aureus*, and staphylococcal enterotoxin.
3. Dried milk products: *Salmonella*, *C. perfringens*, *E. coli*, staphylococcal enterotoxin, *S. aureus*, *C. botulinum*, and *L. monocytogenes* (International Dairy Foods Association, 1998; Food and Drug Administration, 2006).

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Table 13.4. Hazard and control guide for milk plant raw materials (FDA Hazards and Control Guide for Milk Plant Raw Materials (2006)).

Ingredient or process	Type of hazard	Potential hazard	Hazard rationale	Hazard management or controls	Additional resources*
Raw milk	Biological	B-1: Presence of vegetative pathogens	B-1: Scientific studies have shown that a wide range of pathogens (organisms that can cause illness in humans) can be present in unpasteurized milk[1,2]	B-1: Minimize the incoming bacterial load by purchasing grade "A" IMS listed raw milk and testing incoming product. Verify that tank trucks were cleaned and sanitized prior to picking up the milk being unloaded (wash tags or in the case of trucks that only deliver to one plant, plant cleaning records) and that milk has been maintained at the proper temperature	PMO Sec4 PMO item 12p IMS List PMO 17p DPC 25 DPC 50
	Chemical	C-1: Presence of therapeutic drugs	C-1: This hazard must be addressed based on "other NCIMS Requirements"	C-1: At a minimum, screen all tankers for animal drug residues as required by appendix N or other regulatory mandates. In addition, plants are encouraged to screen for other residues as indicated by available information	M-a-75 M-a-86 PMO Appendix N DPC 22
	Chemical	C-2: Presence of mycotoxins	C-2: Mold growth in animal feed can contaminate milk with aflatoxin M1	C-2: Aflatoxin has been shown to be present in raw milk dependent on geographical location, growing season conditions and past history. Other management controls may include supplier guarantees and COAs	
	Physical	P-1: Extraneous material	P-1: If dairy cattle are not kept clean or the milk is drawn in an unclean environment and is not properly protected, the physical objects from the farm environment may become incorporated into the milk	P-1: Not to be included in the hazard analysis if purchasing milk from grade "A" IMS listed sources to minimize the contamination	

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Pasteurized milk, heat treated milk or cream and condensed skim milk	Biological	B-1: Presence of vegetative pathogens	B-1: Heat-treated milk products may not have been heated sufficiently to deactivate these organisms	B-1: Heat-treated milk or cream should be treated as raw milk and come from approved sources	IMS List PMO Sec7
	Biological	B-2: Contamination by vegetative pathogens	B-2: Bulk shipped pasteurized milk products may have been subject to recontamination during transit	B-2: Verify that tank trucks were cleaned and sanitized prior to picking up the milk being unloaded (wash tags or in the case of trucks that only deliver to one plant, plant cleaning records) and that milk has been maintained at the proper temperature	PMO Items 12p, 17p and 21p 3-A SS 605
	Chemical	None			
	Physical	None			
Other ingredients or packaging materials	Biological	B-1: Presence of vegetative pathogens	B-1: Pathogens may be present in ingredients[3,4,5]	B-1: Supplier certificates of analysis	21 CFR 110.80 (a)
	Chemical	C-1: Presence of toxic or carcinogenic substances	C-1: Adulteration with toxic or carcinogenic chemicals has been documented[6,7]	C-1: IMS listed packaging suppliers. Supplier's letter of guarantee	21 CFR 110.80 (a) 21FCR 176.260 21FCR 178.010
	Physical	P-1: Extraneous material	P-1: Free of foreign materials which constitute the food safety hazards[4]	P-1: Supplier letter of guarantee	21FCR 110.80 (a) CPG 555.425

*Abbreviations used in Additional resources column: Pasteurized Milk Ordinance (PMO); U.S. Code of Federal Regulations (CFR); Interpretive Memoranda published by the FDAIMS List, M-a,M-b, and M-I memoranda; IMS List Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers; FDA Compliance Policy Guides (CPG); 3-Sanitary Standards (3-A SS) and 3-A Accepted Practices (3-A AP); Dairy Practices Council (DPC) Guidelines. References are to specific guideline identification numbers, "DPC 8"; Good Agricultural Practices (GAPs); Current Good Manufacturing Practices (cGMPs); National Drug Residue Database (NMDRD) Report.

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Table 13.5. Hazard and control guide for milk plant processing operations, partial list (FDA Hazards and Control Guide for Milk Plant Processing Operations (2006)).

Ingredient or process	Hazard	Type of hazard	Hazard rationale	Hazard management or controls	Additional resources
Receiving materials shipped by bulk tanker, e.g., fluid milk and milk products	Biological	B-1: Contamination with vegetative pathogens	B-1: The truck unloading area has the potential to contaminate liquid milk products. These products are normally transmitted through equipment that if unclean (or uncleanable) can result in bacterial contamination	B-1: Truck unloading area should be constructed to protect the milk (at a minimum overhead protection and concrete, or equivalent surface under the truck that is properly drained). Maintain the truck unloading area and equipment clean. Protect the milk that is being unloaded by closing in the unloading area or using filters over the vent/personnel access port area. Using equipment meeting sanitary design guidelines	DPC 8 PMO Item 5p(4) & 15p(A)(3) 3-A SS 02-, 11-, 28-, 29-, 53-, 58-, 59-, 62-, 63-, 74
	Chemical	C-1: Cleaning and sanitizing residues	C-1: Without proper separation between cleaning and sanitizing solutions and product there could be contamination of the product[1]	C-1: Maintain proper separation or a physical break between circuits containing cleaning solutions and vessels and lines used to contain or conduct product	PMO Item 15p(B)(1) 3-A AP 605 21CFR178.1010(a)
	Physical	P-1: Extraneous materials	P-1: Free of foreign material which constitute food safety hazards[2]	P-1: Use a filter, screen or other appropriate device at some point in the system	3-A SS 10- & 42- PMO Item 11p(8)
	Physical	P-2: Metal shavings, gasket material and other foreign material from receiving equipment	P-2: Equipment in poor repair or improperly assembled may contaminate product with foreign material	P-2: An effective preventive maintenance program and routine (daily) inspection of equipment for wear or missing parts. Use of a filter, screen or other appropriate device at some point in the system	PMO Item 11p 3-A SS 10- & 42-

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Receiving materials shipped by common carrier, e.g., dry ingredients, flavors and packaging materials	Biological	B-1: Contamination with vegetative pathogens	B-1: Product may become contaminated if product containers are damaged during shipment	B-1: Inspect product during unloading operations for damage	DPC 8 21CFR 110.80(a)(2)
	Chemical	C-1: Toxic chemicals	C-1: Delivery trucks may have been used to transport toxic chemicals prior to food products or packaging materials[1]	C-1: Inspect vehicles prior to unloading for evidence of unsanitary conditions, spilled chemicals, off odors, of evidence that might indicate the delivered product may have been contaminated	DPC 8
	Physical	P-1: Extraneous materials	P-1: Vehicles may not have been maintained in good repair or have been used to carry metal or wood articles	P-1: Inspect vehicles prior to unloading for evidence of foreign materials that may have contaminated the product	DPC 8
Raw milk storage	Biological	B-1: Contamination with vegetative pathogens	B-1: These products are normally stored in vessels that, if unclean (or uncleanable) can result in bacterial contamination[1]	B-1: Verify that storage vessels and associated lines and valves and similar appurtenances are constructed in such a way that they can be cleaned. Maintain records that storage vessels are cleaned after each use. Maintain records that the associated lines, valves and similar appurtenances are cleaned as needed but at least each day used. Pipeline openings (e.g., flow control panels) and outlet valves are capped when not in use, other openings are closed with tight fitting covers. Associated pipelines and similar appurtenances are similarly protected	PMO Item 12p 3-A SS 22- & 63- 3-A AP 605 21CFR 110.35(d) PMO Item 15p(A)(3)

(*Continued*)

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Table 13.5. (*Continued*)

Ingredient or process	Hazard	Type of hazard	Hazard rationale	Hazard management or controls	Additional resources
	Biological	B-2: Growth of vegetative pathogens	B-2: Without proper temperature and time controls, vegetative pathogens can multiply to levels that may be capable of overwhelming the pasteurization process without proper temperature and time controls[1]	B-2: Maintain the temperature sufficiently low to minimize the growth of pathogens. Clean the storage vessels and associated lines and valves and similar appurtenances at frequencies that do not allow for bacterial growth of pathogens in the product at the product temperature used. *Note*: If times or temperatures less stringent than specified in the PMO are used, they must be reviewed and found acceptable to the State and FDA	PMO Item 17p PMO Item 12p 21CFR 110.35(d) PMO Item 12p
	Chemical	C-1: Cleaning and sanitizing solution residues	C-1: Without proper separation between cleaning and sanitizing solutions and product there could be product contamination[1]	C-1: Maintain proper separation or physical break between circuits containing cleaning solution and vessels and lines used to contain product	PMO Item 15p(B) 3-A AP 605 21CFR 178.1010(a)
	Physical	None			

See footnote to Table 13.4 for explanation of abbreviations used in Additional resources column.

[1]Lund, B.M., Baird-Parker, T.C. & Gould, G.W. (eds) (2000) *The Microbiological Safety and Quality of Food*, Vols I and II. Aspen Publishers, New York.

[2]Roberts, T.A., Pitt, J.I., Farkes, J. & Grau, F.H. (eds) (1998) *Microorganisms in Foods 6: Microbial Ecology of Food Commodities*. International Commission on Microbiological Specifications for Foods, Blackie Academic & Professional Publishers, London.

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The possible chemical hazards involved are as follows:

1. Raw milk: antibiotics, pesticides and sulfonamides.
2. Cheese: nitrates, nitrites, pesticides and aflatoxin.
3. Dried milk products: sulfonamides, pesticides and antibiotics (International Dairy Foods Association, 1998; Food and Drug Administration, 2006).

The possible physical hazards involved in all dairy products include insects, soil, glass and wood fragments, hair and plastics.

13.6 RECOMMENDED CONTROL SYSTEMS FOR PRODUCTION OF QUALITY MILK PRODUCTS

To secure control systems for the production of quality milk and dairy products, it has been recommended that a Dairy HACCP Safety System be established at each commercial dairy plant or individual dairy processing facility. It has been recommended by the FDA and International Dairy Foods Association (IDFA) that the prerequisite programs for effective monitoring and control be instituted before developing an HACCP plan. The HACCP may be implemented only in a facility that is constructed and operated to provide a sanitary environment. Milk plant, receiving station or transfer station premises, building construction, maintenance, and housekeeping shall be maintained in a manner sufficient to provide such an environment. These factors shall be controlled by effective plant, receiving station or transfer station programs or by prerequisite programs, as the plant, receiving station or transfer station chooses (Food and Drug Administration, 2006). Comprehensive and effective prerequisite programs can simplify HACCP plans, and ensure maintenance of the integrity of the HACCP processing plant and the safety of the manufactured product (Park, 2010).

There are seven essential prerequisite areas for developing a comprehensive HACCP plan for a commercial dairy plant as delineated by the International Dairy Foods Association (1998) and Food and Drug Administration (2006):

1. Premises:
   (a) outside property
   (b) building
   (c) sanitary facilities
   (d) water quality program.
2. Receiving/storage/shipping:
   (a) receipt of raw materials, ingredients, and packaging materials
   (b) specifications
   (c) storage
   (d) distribution.
3. Equipment performance and maintenance:
   (a) general equipment design
   (b) equipment installation
   (c) equipment and maintenance.
4. Personnel training program:
   (a) manufacturing control
   (b) hygienic practices
   (c) controlled access
   (d) personnel safety.
5. Cleaning and sanitation:
   (a) cleaning and sanitation program
   (b) pest control program.
6. Recall programs:
   (a) traceability
   (b) recall system
   (c) recall initiation.
7. Supplier control programs:
   (a) performance criteria
   (b) alternative sources.

These areas can also be utilized as the reference guidelines of a quality control program for small farm dairy operations. There are two important areas that require special attention:

1. Outside the property the land ought to be free of debris and refuse, and not in close proximity to any source of pollution (e.g., objectionable odors, smoke, dust or other contaminants). Roadways should be properly graded, compacted, dustproof, and drained. Premises and shipping and receiving areas should provide or permit good drainage.
2. The buildings and facilities should be designed to readily permit cleaning, and prevent entrance and harboring of pests and entry of environmental contaminants (International Dairy Foods Association, 1998; Food and Drug Administration, 2006).

The production of quality milk and dairy products requires hygienic practices. Ongoing training in personal hygiene and hygienic handling of food should be provided to all persons and food handlers entering food handling premises. The major areas of hygienic practices recommended by the International Dairy Foods Association (1998) and Food and Drug Administration (2006) include:

1. communicable diseases;
2. injuries;
3. washing of hands;
4. personal cleanliness and conduct;
5. controlled access; and
6. personal safety.

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13.7 ETIOLOGY OF MASTITIS AND MILK HYGIENE

Mastitis is the inflammatory response of the mammary tissue to physiological and metabolic changes, traumas, and allergies and is almost always caused by infecting microorganisms. Although there may be some overlap between groups, mastitis pathogens may be broadly classified as contagious pathogens (*S. aureus*, *Streptococcus agalactiae*, *Streptococcus bovis*), environmental pathogens (*E. coli*, *Pseudomonas aeruginosa*, *Streptococcus uberis*, *Staphylococcus chromogenes*), and other coagulase-negative staphylococci (CNS). Mastitic infections may be classified as either clinical or subclinical; the former is defined by the IDF as an udder inflammation characterized by visible abnormalities in the milk and udder, and which may be graded as mild, moderately severe and seriously severe. Subclinical mastitis is defined as inflammation that is not visible and requires a diagnostic test for detection, such as measurement of SCC, and is the most prevalent form of the disease. Chronic mastitis refers to cases where the infection is of long duration and may show periodic clinical symptoms.

Mastitis is a highly multifactorial disease and presents different degrees of intensity, duration and consequences. This means that disease manifestations and pathogen patterns are interrelated with a number of animal and environmental factors in a complex way. Soiled litter, feces, water, air, improper milking procedures, and inadequate milking equipment and vacuum are regarded as the main sources of mastitis pathogens.

Because important differences exist among dairy ruminants, the approach to mastitis control in sheep and goats should be species-specific and not by generalizing results obtained from research on mastitis in dairy cows (Contreras *et al*., 2007). Handling could be important in the predominance of certain bacterial species and in the etiology and epidemiology of mastitis. This might explain differences in the bacterial species that are prevalent in dairy herds as well as in intensively or extensively managed herds. The gradual spread of intensive production systems, as a consequence of the increased size of specialized dairy flocks and herds, is a development that demands more information about the prevalence and etiology of udder infection on such intensively managed farms. Albenzio *et al*. (2002) found that environmental pathogens, primarily *E. coli*, were largely predominant in the milk from infected ewes in intensively managed flocks. A relatively low frequency of contagious pathogens was observed, suggesting that in intensive production systems udder health may benefit from efficient sanitation control of milking personnel and equipment.

Other pathogens such as *Streptococcus* spp., Enterobacteriaceae, *Ps. aeruginosa*, *Mannheimia haemolytica*, corynebacteria and fungi cause IMI in small ruminants. In addition, severe cases of mastitis related to incorrect preventive strategies have been attributed to the pathogens *Aspergillus fumigatus* and *Serratia marcescens*. Lentiviruses are also known to infect goats and sheep, but because they rarely produce clinical symptoms or elevated milk SCC, they are not usually considered as classic small ruminant intramammary pathogens. Nevertheless, caprine lentiviruses should still be included in the general plan for controlling mastitis (Contreras *et al*., 2003). In herds clinically infected by *Mycoplasma* spp., besides significant losses due to mortality or the need to cull animals, producers cannot comply with the milk quality standards demanded by consumers, industry and public health organizations (Corrales *et al*., 2004).

Rather than risk a threat to human health that could be caused by some mastitis-causing bacteria, milk is generally heat treated to minimize this effect. However, in regions where cheese is made from raw milk, controlling clinical and subclinical mastitis becomes a priority. Because of its zoonotic importance, preventing milk contamination by *L. monocytogenes* is a high priority for the industry (Contreras *et al*., 2007). Although most cases of milk-borne listeriosis are related to spoilage of the raw milk through fecal or environmental cross-contamination, few cases of *Listeria* mastitis have been reported in sheep. One report of clinical mastitis caused by *L. monocytogenes* described a highly increased SCC and persistent shedding of bacteria through milk (Winter *et al*., 2004). Similarly, severe human infections attributed to the consumption of non-pasteurized cow milk were associated with mastitis caused by *Streptococcus zooepidemicus* (Balter *et al*., 2000). There have also been descriptions of mastitis due to *S. zooepidemicus* in goats and sheep (Las Heras *et al*., 2002).

The identification of *Nocardia* spp. has also been considered important, due to their potential for causing disease in humans, and because *Nocardia farcinica* is known to cause mastitis in goats (Maldonado *et al*., 2004). *Nocardia farcinica* is a significant public health concern owing to its aggressiveness, its tendency to disseminate, its resistance to antibiotics, and its laborious biochemical identification. These difficulties could have contributed to the increased incidence of disease caused by this microorganism in developed countries (De La Iglesia *et al*., 2002). IMIs caused by *S. aureus* warrant special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and subclinical mastitis. *Staphylococcus aureus* secretes several toxins that contribute to the pathogenesis of mastitis and also plays a role in food-borne

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disease, even with pasteurized milk because of the thermostable enterotoxins. These enterotoxins are produced not only by *S. aureus* isolates from clinical mastitis but also by isolates from subclinical mastitis. In this sense, De Santis *et al*. (2005) found that *S. aureus* isolates in milk from sheep with subclinical mastitis are less enterotoxigenic than isolates from sheep with acute clinical mastitis. Because of the production of these thermostable enterotoxins from *S. aureus* isolates, a main priority should therefore be the implementation of programs to eradicate *S. aureus* from dairy flocks.

Under European legislation, the control of *S. aureus* is mandatory, such that the marketing of sheep, goat and cow milk containing *S. aureus* is highly restricted. In addition to enterotoxins produced by *S. aureus*, there is also a wide pattern of virulence factors, such as the leukotoxins which can selectively kill host PMNs and monocytes. In an investigation on the leukotoxic actions of *S. aureus* strains isolated from cows, sheep and goats with mastitis, Rainard *et al*. (2003) found that most isolates were leukotoxic and that strains isolated from small ruminants were more leukotoxic toward bovine PMNs than strains of bovine origin. Besides producing toxins, *S. aureus* also secrete exopolysaccharides (slime), which form a protective barrier that restricts the efficiency of both the host immune response and chemotherapy (Baselga *et al*., 1994). The best strategy for controlling IMI by *S. aureus* is to remove infected animals from the herd along with conventional precautions such as milking hygiene and dry therapy.

Although less pathogenic than *S. aureus*, CNS can produce persistent subclinical mastitis and a significant increase in SCC of milk. CNS can also lead to clinical mastitis and the production of thermostable enterotoxins (Meyrand *et al*., 1999). Despite the accepted role of these bacteria as major IMI-causing pathogens in small ruminants, the pathogenicity of the different CNS species varies widely. The most commonly isolated CNS species in persistent subclinical IMI in goats and sheep are *Staphylococcus epidermidis*, *Staphylococcus caprae*, *Staphylococcus simulans*, *Staphylococcus chromogenes* and *Staphylococcus xylosus* (Gonzalo *et al*., 2002). *Staphylococcus epidermidis* and *S. caprae* are among the most prevalent causal microorganisms in goats and *S. epidermidis* and *S. simulans* are prevalent in ewes. The presence of different CNS species could be ascribed to certain practices for controlling mastitis, such as the protocol and type of disinfectant used for teat dipping or dry-off treatments. Despite the high incidence of CNS linked to IMIs in sheep and goats, the pathogenic mechanisms that underlie subclinical infections remain largely unknown.

Using *S. epidermidis* to induce IMI, Winter and Colditz (2002) observed that lactating udders are capable of a prominent local inflammatory response; cytokine levels were significantly elevated soon after infection, peaking between 8 and 24 hours, and increased interleukin (IL)-1 levels persisted for 144 hours. In parallel, SCC peaked at 8 hours but counts returned to normal values between 48 and 144 hours, despite the presence of bacteria in milk. These authors suggested a complex relationship between cytokines and the course of infection, because cytokines and PMNs decreased as infection progressed. Surface adhesion molecules play a role in PMN adherence to, and migration through, the endothelial lining of the vascular wall. Cytokines play a critical role in PMN recruitment to inflamed tissue; these soluble cell-derived molecules influence cell responses by different ways, such as adhesion molecule expression, by binding to cell surface receptors and activating intracellular signal transduction pathways leading to transcriptional activation. Two well-described proinflammatory cytokines, tumor necrosis factor (TNF)-α and IL-1β, induce vascular endothelial adhesion molecule expression, thereby promoting PMN transendothelial migration to the site of infection.

Another cytokine involved in PMN recruitment is IL-8, which is directly chemotactic for PMNs (Wagner & Roth, 2000). Experimental IMI of sheep with *S. aureus* and *E. coli* has been shown to induce a significant increase in milk leukocytes within 24 hours of infection (Persson-Waller *et al*., 1997). Similar to cattle (Bannerman *et al*., 2004), *E. coli* IMI elicited more rapid recruitment of PMNs to the gland than *S. aureus*. Interestingly, this delay in PMN recruitment in response to *S. aureus* in both cattle and sheep correlated with impaired clearance of *S. aureus* compared with that of *E. coli*. Maximal increases in milk levels of ovine TNF-α and IL-8 preceded or were temporarily coincident with maximal PMN recruitment to glands after infection with pathogens (Persson-Waller *et al*., 1997). Direct evidence supporting this notion has been provided in studies investigating the direct effects of these cytokines on changes in PMN levels in lactating ovine glands (Persson *et al*., 1996). Infusion of ovine IL-1β or TNF-α into either the teat cisterns or udders of sheep induced an increase in leukocytes, the majority of which were PMNs. Infusion of ovine IL-8 into the teat cistern, but not the udder, elicited an increase in PMNs. The identification of cytokines and adhesion molecules responsible for PMN recruitment has deepened our knowledge of the mechanisms by which elevations in SCC occur during IMI.

13.8 CELL TYPES AND COMPOSITION OF MILK IN RESPONSE TO MAMMARY GLAND INFLAMMATION

In addition to determining the different types of immune cells present in milk, the total SCC is useful for describing

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udder health status. In the mammary gland, the number and distribution of leukocytes are important for successful udder defense against invading pathogens. Lymphocytes, macrophages, and PMNs play an important role in inflammatory responses within the mammary gland. PMNs are phagocytic cells that have a specific bactericidal function. They possess an arsenal of enzymes and chemical agents that can destroy engulfed bacteria. Macrophages are also phagocytes and can ingest bacteria, cellular debris, and accumulated milk components, whereas lymphocytes are responsible for immune memory and antibody production.

Milk or tissue macrophages recognize the invading pathogens and initiate an immune response, inducing the rapid recruitment of PMNs into the mammary gland. The main task of PMNs is to defend against invading bacteria at the beginning of an acute inflammatory process, when not only the number of PMNs increases enormously but their defensive responses also increase.

In normal milk from uninfected quarters the SCC is typically below 100 000 cells/mL. Non-pathological factors such as breed, parity, stage of lactation, estrus, and diurnal, monthly and seasonal variations can contribute to changes in the SCC of milk from dairy cows, sheep and goats. According to Bergonier *et al*. (2003), in ewes non-pathological factors are responsible for geometric mean variations ranging from 40 000 to 250 000 cells/mL. Some investigations have demonstrated that goat milk from healthy udders contain 50 000–400 000 cells/mL throughout lactation; however, it has also been reported that milk from healthy goats can contain even several million cells per milliliter, especially in later stages of lactation (Haenlein & Hinckley, 1995; Bagnicka *et al*., 2011).

Increases in milk SCC during IMI are an essential part of mammary gland defense against an invading pathogen. The early increases in milk somatic cells are primarily due to the recruitment of circulating PMNs from the blood to the inflamed tissues. Once PMNs have migrated to the gland and become activated, they release a number of antibacterial components that are essential for successful host clearance of the infectious pathogens. Recruitment of PMNs to the gland occurs through a process referred to as chemotaxis. Chemoattractants are soluble molecules secreted from inflamed tissue that enable directional migration of PMNs to the site of infection. In addition to chemoattractants, PMN chemotaxis requires the expression and interaction of complementary adhesion molecules on PMNs and endothelial cells. Endothelial cells line the luminal surface of the vascular wall and regulate leukocyte trafficking (Wagner & Roth, 2000).

The major role attributed to the SCC as a milk quality criterion depends on the fact that leukocyte count is a reliable indicator, but only in cow milk, of both milk hygienic quality and nutritional properties. Indeed, milk of animals suffering from clinical or subclinical mastitis displays a dramatic reduction in lipid, protein and lactose contents and upward change in mineral contents compared with milk from non-infected animals.

Albenzio *et al*. (2002) carried out a survey on etiology of mastitis in dairy sheep flocks and related changes in ewe milk and categorized milk samples from animals by type of pathogen (contagious, environmental, CNS) and mastitis feature, for example infectious mastitis (bacteriologically positive milk samples with SCC >1 000 000 cells/mL), subclinical mastitis (bacteriologically positive milk samples with SCC <300 000 cells/mL), and non-mastitis (bacteriologically negative milk samples with SCC >1 000 000 cells/mL). These authors observed a 7–23% reduction in milk yield from infected animals compared with non-infected ones, while protein content decreased by 10–19%, casein by 14–21%, fat by 3–16%, and lactose by 10–13%. Various types of pathogens had a different impact on milk yield and composition, whereas most reduction in milk yield and deterioration of milk quality grew with the magnitude of SCC increase, being more marked in infectious and non-specific than in latent mastitis cases. Hence, the survey confirmed that enzymes produced by leukocytes play a major role in the worsening of the milk nutritional profile by damaging epithelial secretory cells via the same mechanisms used to face invading pathogens.

IMI also induces a rise in the permeability of the endothelium to the passage of components from blood to milk and the activity of endogenous enzymes. Endogenous proteolytic enzymes, such as the plasmin–plasminogen system, elastase and cathepsin, are mainly associated with leukocytes and are involved, to different degrees, in milk casein breakdown. The increase in these enzymes during IMI is linked to an increase in PMNs and could be explained by two mechanisms: (i) the proteases can be released by vesicles like lysosomes from PMNs or (ii) caseins or fat globules can be endocytosed by PMNs with the degradation products being released into the milk after intracellular digestion. The order in which caseins are degraded by leukocyte proteinases is as follows: α_{s1}-casein, followed by β-casein and to a lesser extent κ-casein and α_{s2}-casein (Napoli *et al*., 2007).

Plasmin is the main native proteolytic enzyme in milk and is part of a complex system in milk. The plasmin system derives from blood where it is involved in the degradation of fibrin clots; in milk it is associated with the casein micelles. As with plasmin, cathepsin D, an acid

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aspartic proteinase, is part of a complex system; the major type in bovine milk is the inactive procathepsin D. The level of cathepsin D in milk is correlated with SCC and is associated with milk PMNs and macrophages. Elastase is a neutral serine-type proteinase mainly associated with PMNs. It is worth noting that the increase in milk SCC causes an increase in the amount of milk proteolytic activity, which in turn reduces the milk yield and nutritional quality. Proteolysis in milk prior to cheese manufacturing is associated with alterations of cheesemaking properties, i.e., longer coagulation time and weak coagulum, leading to poor syneresis, increased moisture content, lower cheese yield, and lower fat contents (Pirisi *et al*., 2000; Albenzio *et al*., 2004a, 2005). Given that there is an increasing demand for cheeses, knowledge of the role of endogenous proteolytic and lipolytic enzymes on cheesemaking ability is needed.

In bulk milk the increased conversion of inactive zymogen (plasminogen) to plasmin can result from the increased production of urokinase-type activators by macrophages and neutrophils. PMNs were found to be responsible for intense proteolysis in ewe milk samples (Albenzio *et al*., 2009) whereas macrophages were found to minimally contribute to the proteolytic activity in ewe milk (Caroprese *et al*., 2007). Individual ewe milk with high SCC (>1 000 000 cells/mL) shows increased proteolytic activity (Albenzio *et al*., 2004a, 2005). Positive correlations have been observed between plasmin and SCC of ewe milk samples at very low SCC (175 000 cells/mL) (Castillo *et al*., 2008). A recent study (Albenzio *et al*., 2011) reported that plasmin activity was highest in ewe milk samples with SCC above 2 000 000 cells/mL and lowest in milk samples with SCC below 300 000 cells/mL. The differences in plasmin activity observed among SCC classes evidences a mild activation of plasmin as the SCC of milk rises. The downregulation of milk secretion is associated with mild activation of plasmin, with an increase in enzyme activity of 10–40%. Plasmin activity displays an increase of about 30% in milk samples with SCC ranging from <300 000 up to 1 000 000 cells/mL, and of about 43% with SCC above 1 000 000 cells/mL.

Udder health also affects the activities of the plasmin system in caprine milk; plasmin activity from infected udders (SCC >1 500 000 cells/mL) has been found to be almost double that of milk with low SCC (<500 000 cells/mL). Casein proteolysis occurs spontaneously in milk during lactation of goats, and somatic cell-mediated conversion of plasminogen to plasmin is an important local factor in further promoting proteolysis of casein and possibly mammary tissue protein. The complexity of the factors regulating the plasmin system in goat milk has been reported to explain the lack of positive correlation between plasmin and SCC in goat milk (Albenzio *et al*., 2006). In goat milk, cathepsin D was not found to be correlated with SCC as a consequence of low SCC in milk (<300 000 cells/mL); conversely, elastase activity was found to be correlated with SCC as a consequence of the origin of this enzyme (Santillo *et al*., 2009).

13.9 FLOW CYTOMETRIC METHOD FOR LEUKOCYTE DIFFERENTIAL COUNT

Together with SCC, determination of the differential cell count in milk is an important tool for detecting the proportion of leukocyte cell types during various phases of inflammation and represents a useful approach for evaluating the immune status of the mammary gland. The first method for enumerating and differentiating somatic cells in milk is direct microscopic differential count. This method, although slow and labor-intensive, remains in many instances the reference method against which other methods are calibrated. Flow cytometry is a routine test for the rapid discrimination of leukocytes in milk and is used to differentiate leukocyte cell types in cow, sheep and goat milk (Koess & Hamann, 2008; Albenzio & Caroprese, 2011; Boulaaba *et al*., 2011). This method is based on differential SCC by fluorescence properties, using monoclonal antibodies against CD molecules or DNA labeling, and by ordering the shapes of cells into clusters that can be directly related to cell types. In cow milk the use of a flow cytometric dot plot to differentiate cells and to determine the percentages of cell types is well documented and recently reviewed by Schwarz *et al*. (2011). In cow milk, lymphocyte proportions range between 20 and 30%, macrophage proportions between 60 and 70%, and PMNs between 10 and 30%. In milk from cows with mastitis, the proportion of PMNs can reach 95% (Kehrly & Shuster, 1994; Kelly, 2002).

In ewe milk samples, flow cytometry was used to detect the percentage of PMNs, macrophages, and lymphocytes in bulk milk and in individual milk samples with different SCCs (Albenzio *et al*., 2009, 2011; Albenzio & Caroprese, 2011). The small number of somatic cells in milk from healthy animals makes the identification of leukocytes more difficult using the microscopic differential cell count. Albenzio and Caroprese (2011) described the flow cytometric method for differential leukocytes in individual ewe milk samples with low SCC (<300 000 cells/mL) and high SCC (>1 000 000 cells/mL). These authors firstly compared the flow cytometry method with the direct microscopic count (standard method) and did not find differences between the two methods for the detection of macrophages, lymphocytes, and PMNs in ovine milk with low and high SCC (Table 13.6). Furthermore, positive correlations were

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Table 13.6. Differential cell count (SCC) of lymphocytes, PMNs, and macrophages using direct microscopic differential leukocyte count (MDLC) and flow cytometry (FC) in ewe milk samples with SCC <300 000 cells/mL (LSCC) and SCC >1 000 000 cells/mL (HSCC).

	SCC level	MDLC	FC	SEM	*P*-value
Lymphocytes (%)	LSCC	53.56[b]	51.34[b]	2.65	<0.001
	HSCC	39.11[a]	38.26[a]		
PMNs (%)	LSCC	42.34[a]	40.17[a]	2.2	<0.001
	HSCC	55.48[b]	57.32[b]		
Macrophages (%)	LSCC	7.07	7.73	2.00	NS
	HSCC	5.4	5.09		

a, b Indicates level of significance in rows.

NS, not significant; SEM, standard error of the mean.

Source: reproduced from Albenzio & Caroprese (2011), with permission of Cambridge University Press.

found for lymphocytes, PMNs, and macrophages using the microscopic and cytometric methods in both low and high SCC samples (Albenzio & Caroprese, 2011). Lymphocyte count ranged between $273.91 \pm 56.62 \times 10^3$ cells/mL and $308.90 \pm 46.15 \times 10^3$ cells/mL in low and high SCC, respectively.

The absence of differences in lymphocyte count between low and high SCC milk showed that in ewe milk this population is quite stable and not influenced by changes in total SCC. This finding supports the hypothesis that in ewe milk with low and high SCC, lymphocytes are not recruited into the ewe mammary gland in response to inflammation, suggesting that resident lymphocytes may be able to mount the immune response.

PMN number was lower in low compared with high SCC milk ($248.83 \pm 46.87 \times 10^3$ cells/mL vs. $444.38 \pm 58.62 \times 10^3$ cells/mL); accordingly PMN percentage was lower in low SCC milk (40%) than in high SCC milk (57%). The positive correlation between PMNs and SCC in ewe milk samples shows that PMNs may be considered a good marker for evaluating ewe udder health. In ewe milk with SCC below 300 000 cells/mL, PMNs were positively correlated with non-viable PMNs ($r = 0.75$; $P < 0.001$), suggesting that resident PMNs in low SCC milk are not recruited to play a protective role in the ewe mammary gland. Conversely, PMNs are not significantly correlated with non-viable PMNs in milk samples with SCC above 1 000 000 cells/mL, demonstrating that the recruitment of defense cells is activated in response to immune stimuli. Mehrzad *et al*. (2004) reported that the PMNs recruited in the udder during the transition from normal to high SCC milk are relatively young and show slow apoptosis, while the PMN population resident in the udder is old and not very efficient. Resident PMNs in cow milk with low SCC modulate the initial steps of dynamic immune defense of the udder (Mehrzad *et al*., 2004).

Flow cytometry also shows that the percentage of macrophages is about 7% in milk with SCC below 300 000 cells/mL and 5% in milk with SCC above 1 000 000 cells/mL, showing that macrophages are not the predominant class of leukocytes in ewe milk and did not vary in milk samples with low and high SCC (Albenzio *et al*., 2011). Conversely, in cow milk, macrophages are the predominant cells from a healthy udder (Kelly & Fox, 2006) and normal bovine milk from uninfected quarters with SCC below 100 000 cells/mL contains 60–70% macrophages (Kehrly & Shuster, 1994; Kelly, 2002).

Generally, in goats free of IMI, PMNs constitute 45–74% of milk somatic cells and 71–86% in milk from infected mammary halves. Macrophages comprise 15–41% of somatic cells in uninfected halves and 8–18% in infected halves. Lymphocytes comprise 9–20% of somatic cells in uninfected halves and 5–11% in infected halves. Epithelial cells are low in goat milk, but identification by light microscopy is difficult because of the presence of cytoplasmic particles in goat milk. Milk secretion in the goat is apocrine: cytoplasmic particles are shed into milk from the apical portion of the mammary secretory cells. The numbers of cytoplasmic particles in milk from uninfected mammary halves ranges from 71 to 306 000 cells/mL, and from 98 to 231 000 cells/mL in milk from infected mammary halves. Although the majority of these particles are generally anucleated, approximately 1% contain nuclear fragments. In ewe colostrum and ewe milk, cytoplasmic particles are found in a low percentage whereas they average 15 000 cells/mL in goat milk (Martinez *et al*., 1997). PMNs are the most numerous cell type in milk from infected and uninfected mammary glands of goats, which is different from cow milk where macrophages are the predominant cell type. Flow cytometric studies of somatic cells in goat milk have focused on cell subpopulations like T helper cells, regulatory T cells, γδ T cells, and B cells (Davis *et al*., 2007). Boulaaba *et al*. (2011) applied a flow cytometric dot plot to generate a quick differential cell count in goat milk. This study showed how to use flow cytometric dot plots to construct quick differential cell counts from goat milk in a way similar to that for bovine milk. However, it was necessary to employ DNA-specific fluorescent dyes in order to avoid overlapping of somatic cells and cytoplasmic particles. At present, there are few other studies dealing with differential cell counts of goat milk, their changes in relation to udder health, and suitable

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methods for detection. This is largely due to the complex and labor-intensive techniques that have been used for cell differentiation.

13.10 FACTORS AFFECTING MILK COMPOSITION AND YIELD IN RELATION TO MILK QUALITY

There are a number of factors which affect milk quality standards. Some of them exert their effects during milk synthesis and secretion, others when milk is processed for dairy products. It has been shown that composition and yield of milk can be influenced by many factors including for example diet, breed of animal, stage of lactation and environmental temperature.

Bacterial load and SCC in milk are the outcome of a balance between the number and pathogenicity of microorganisms coming into contact with the teat and the integrity and reactivity of animal's immune system. Therefore, the main role in increasing the bacterial load and SCC in milk is played by poor housing and milking hygiene, and exposure of dairy animals to stressful challenges that impair their immune function. The major factors affecting composition and yield of milk as related to the quality of milk are listed in Table 13.7, and are further delineated in the following sections.

13.10.1 Diet

The composition of any species' milk is affected by diet, which appears to be similar to the situation for cow milk, although some variations between species have been observed (Juárez & Ramos, 1986). Merlin *et al*. (1988) reported that type of feed affected the total protein content of milk, whereas fat content was not changed. An increased dietary energy content for high-producing goat breeds during lactation was shown to augment milk production and reduce the fat content (0.2–0.4%) while increasing the nitrogen content (0.1–0.15%) (Juárez & Ramos, 1986). Water deprivation for 48 hours in milking goats resulted in reduction of milk yield and higher lactose and protein contents (Dahlborn, 1987).

Calderon *et al*. (1984) observed the role of dietary crude fiber levels on milk fat depression in goat milk. Restricting roughage and providing high levels of concentrates decrease the level of dietary fiber. At least 17% crude fiber in the diet of cows is required to prevent a depression in milk fat (Schmidt, 1971). Morand-Fehr and Sauvant (1980) in a review of the effects of nutritional manipulation on composition and yield of goat milk showed that goats fed a high-concentrate diet underwent nearly a 20% increase in milk yield, with a slight decrease in fat content and increases in lactose and protein content. Thus depression of milk fat content in dairy goats is similar to that in dairy cows.

Table 13.7. Factors affecting composition and yield of milk.

Species
Breed
Individual animal
Stage of lactation
Colostrum
Feed (diet), plane of nutrition
Season
Environmental temperature and humidity
Ventilation
Milking machine
Diseases
Gestation and length of dry period
Age and body weight at kidding
Stocking density
Parity
Genetic polymorphism

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Low-fat diets resulted in a drop in milk fat content, while adding protected lipids led to an appreciable increase in milk fat content (Morand-Fehr & Flamant, 1983). On the other hand, high-protein diets did not change the nitrogen content of goat or cow milk (Vignon, 1976). Higher protein supplementation of the diet above the normal recommended standards had no effect on milk yield but caused a slight increase in the non-protein nitrogen content of cow milk (Juárez & Ramos, 1986).

13.10.2 Breed

Researchers have shown that the breed of milking animal has a significant effect on the yield and composition of milk, both between species and within species. For example, among the Swiss goat breeds the Saanen produces a large amount of milk but which contains somewhat low fat levels, so that it is known as the Holstein of the goat world (Haenlein & Caccese, 1984). The other extreme is the Nubian goat breed, which is equivalent to the Jersey breed of cow. Milk yield of the Nubian is lower, with higher levels of solids, including fat and solids not fat (SNF). The Toggenburg, LaMancha, Oberhasli, and Alpine goat breeds produce milk with a yield and composition between that of the Saanen and Nubian (Haenlein & Caccese, 1984). Similar differences have been documented for other goat breeds in Italy, Spain, Greece, Germany, and India, as well as for breeds of sheep, cows, buffaloes, and camels.

Variation in fat content among different dairy goat breeds is greater than variation in protein content (Ramos & Juárez, 1981). The fat and total solids content of the milk

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of imported Alpine, Saanen and Anglo-Nubian breeds in tropical environments have been shown to be lower than those of the same breeds raised under temperate climate conditions, which might be attributed to both improper diet and the higher temperatures (Juárez & Ramos, 1986). Milking goats of indigenous breeds such as the West African Dwarf and Red Sokoto reportedly have a much richer solids composition but lower yields compared to those of the Swiss breeds.

13.10.3 Stage of lactation

Milk yield and composition are significantly affected by stage of lactation of the dairy cow and all other species. Le Jaouen (1987) showed that a relatively high level of milk production in dairy goats starts at kidding and continues to increase to a peak approximately 3-4 weeks after freshening; a similar trend occurs in dairy cows, with a peak at 4–8 weeks (Schmidt, 1971). High milk yield may be maintained for some weeks, but then milk production gradually (depending on rate of persistency influenced by new pregnancy, diet and environmental temperature) declines toward the end of lactation.

The fat, SNF, and protein content of cow milk are high in the early stage of lactation, decrease during the second to third months of lactation, and then increase toward the end of lactation (Schmidt, 1971; Park & Guo, 2006). There is an inverse relationship between the levels of these components and the yield of milk (Park & Guo, 2006; Park, 2010). In goat milk, fat and protein content decrease from the onset of lactation to the fourth or fifth month and then remain for a variable length of time, increasing at the end of lactation. During different stages of lactation, lactose content in both goat and cow milk shows fluctuations which are opposite to the trend of fat and protein content and yield (Renner, 1982; Larson, 1985; Park, 2010).

13.10.4 Season

Depending on the season, milk fat and SNF content in goat and cow milk may vary by as much as 2% and 1%, respectively (Park & Guo, 2006; Park, 2010). In temperate climates, late summer milk contains the lowest fat and SNF levels (Chandan *et al*., 1992). These seasonal deviations in fat and protein content of milk led a Canadian study to demonstrate that there was a direct reflection of these variations in cheese yields (Irvine, 1974).

Milk composition is also influenced by a different kidding season. Natural breeding usually results in goats freshening at the beginning of spring, although artificial breeding methods are practised for year-round milking. Seasonal variations in milk composition are concomitantly involved with lactation stages in overall observed fluctuations of milk constituents (Chandan *et al*., 1992). The availability of fresh forages changes during different seasons, and this also has significant effects on milk composition and yield.

13.10.5 Environmental temperature

Milk composition and yield are also influenced by environmental temperature, and this manifests differently among species and among breeds. In dairy cows, Holsteins and other large breeds are somewhat more tolerant of lower temperatures, whereas the smaller breeds, especially the Jersey, and to some extent the Brown Swiss, are more tolerant of higher temperatures (Schmidt, 1971). Low environmental temperatures do not have significant effects on milk yield if extra feed is provided to cover the extra energy required to maintain body temperature. Milk production is not affected by temperature changes between 5 and 22°C (40–70°F), when the relative humidity is 60–80%; this is especially true for Holstein cows (Schmidt, 1971).

If the temperature is above the range of thermal neutrality, a considerable decrease in milk production occurs with increase in environmental temperature. At high temperatures, feed consumption decreases and water consumption increases. Feed consumption and milk production approach zero at about 40°C (105°F) (Schmidt, 1971). If the temperature is below 25°C (75°F), the milk fat percentage increases, and the SNF and total solids follow the same trend. If environmental temperatures become high, the chloride content in milk increases while the lactose content decreases. Dairy cows are considered to be less tolerant to environmental temperature changes than dairy goats, Zebu dairy cattle or water buffaloes, but few comparative studies have been reported on the effects of environmental temperatures on milk yield and composition of different breeds and species.

Low and high ambient temperatures adversely affect milk quality standards both directly and indirectly. The exposure of lactating animals to temperatures near 0°C induces local cooling of the udder and a drop in blood flow with a subsequent reduction in oxygen uptake. Such a situation predisposes teats to injuries and trauma, increasing the penetration of microorganisms and the risk of mastitis. Conversely, high ambient temperature, especially when combined with high relative humidity, accelerates decomposition and fermentation of feces and promotes growth and multiplication of microorganisms in the litter, increasing the number of bacteria in contact with the teats. These events are accompanied by a stressful impact of temperature extremes on lactating animals because of the dramatic energy imbalance due to the thermoregulatory efforts made by the animals. Experiments conducted by Sevi *et al*. (2001a) on lactating ewes have shown a significant reduction in immune response together with a marked worsening

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of milk hygienic quality, and an increase in PMNs, staphylococci, coliforms and *Pseudomonas* counts under hot climates.

13.10.6 Ventilation

Ventilation plays a major role in sustaining milk quality standards by removing aerial pollutants, which originate from animals and their excreta. In particular, low ventilation rates can lead to increased relative humidity and higher air concentrations of ammonia and carbon dioxide that may be ascribed to inefficient removal of the moisture and gases that originate from the respiratory activity of animals and the decomposition and fermentation of manure. Conversely, very high ventilation rates can result in higher dust concentrations, probably due to reduced humidity levels and to turbulent air currents maintaining suspension of dust particles in the air for a longer time. In summer, a ventilation rate of about 70 m^3/hour is needed for the lactating ewe (Sevi *et al*., 2002) and about 300 m^3/hour for a dairy cow (Chiumenti, 2004). Lower ventilation rates lead to higher microbial loads and SCCs in milk. During the winter season, low ventilation rates induce a worsening of milk quality in terms of increased SCC and bacterial load, probably due to poor control of air and surface hygiene. However, so also do very high ventilation rates, probably because turbulent air currents encourage the suspension of dust particles and airborne microorganisms (Albenzio *et al*., 2004b).

Airspace has been recognized as one of the most important factors that influence the concentration of airborne particulates in animal houses. In housed calves, doubling the airspace allowance results in a reduction in airborne microorganisms equivalent to that achievable by quintupling the rate of air change. Recommendations for airspace are 18 m^3 per animal for dairy cows (Webster, 1988) and 7 m^3 per animal for dairy sheep (Sevi *et al*., 2001b). Evidence exists that relative humidity increases in animal houses and air quality deteriorates as the volume allotted to animals decreases, suggesting that airspace has direct and indirect effects on air and surface hygiene in animal houses (Sevi *et al*., 2001b). The direct effect is that, other things being equal, the cleanliness of the air with regard to airborne microorganisms and dusts is proportional to the volume of air into which those wastes are dispelled. The indirect effect is that reduced airspace results in increased moisture content of the air and condensation on internal surfaces, both events enhancing the growth and multiplication of microorganisms in the air and the litter. The gradual increase observed in the microbial content of milk with reduction in housing airspace suggests a causal relationship. This indicates that increased microbial concentrations in the bedding may be regarded as the most insidious effects of inadequate volume allocation, especially for small ruminants whose udders are closer to the ground and which may be more affected by the degree of litter pollution than those of cows. The greater bacterial load in milk can account for increased SCC and number of subclinical mastitis cases observed in animals housed with reduced airspace.

13.10.7 Milking machine

Milking management is a critical point on dairy farms. Milking routine, proper handling of animals, and milking hygiene can markedly affect animal health and milk quality standards. Exposure to emotional or physical stress prior to and during milking reduces milk release and depresses immune function, thus increasing the risk of udder disease. Machine milking does not have relevant effects on milk yield or on milk protein and fat content in comparison to hand milking. If machine milking is properly performed, it can improve udder health and the hygienic quality of milk, as demonstrated by reduction in SCC and bacterial counts. However, over-milking, malfunction of the plant system, and poor hygiene in milking operations can have negative effects, above all on the hygienic characteristics of milk. In particular, poor hygiene of milking personnel, the milking machine and milking room is an important cause of udder and milk contamination.

Malfunction of the milking system, due to incorrect installation, lack of maintenance or improper use, can cause animal stress during milking and mammary gland diseases. Vacuum level, pulsation and milking units are the main elements of the milking system. They are closely related to each other and influence milk ejection. These three factors must be well balanced in order to assure optimal functioning of the milking system.

As working vacuum increases, milk flow rate increases. This can cause or favor diseases of the mammary gland. In dairy cows, vacuum increase can cause congestion and edema of the teat walls due to dilation of capillary blood vessels (Hamann *et al*., 1993), and also a greater number of open sphincters after machine milking, a higher probability of hyperkeratosis of the teat end (Mein *et al*., 2003), and an increase in stripping milk (Reinemann *et al*., 2001). Vacuum increase and SCC are positively correlated (Fernandez *et al*., 1999; Sinapis *et al*., 1999). Based on the above implications, the working vacuum level should be as low as possible, with the condition that complete emptying of the mammary gland and no increase in milking duration are achieved. A vacuum of 36–38 kPa is generally recommended for low line systems in good operating conditions.

Milk quality standard is affected not only by the working vacuum level but also by its stability. The mean vacuum

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drop between the receiver and the milk line during milking should not be higher than 2 kPa. Vacuum instability is related to an inadequate milking system with regard to construction (e.g., insufficient vacuum reserve and wrong milk-line dimension) and operation (e.g., anomalous pulsation and wrong milking routine). Conversely, a stable vacuum is a clear indicator that the milking machine is functioning properly. Therefore, an objective way of evaluating the technical and managerial competence of the farmer is to periodically control vacuum fluctuations in the milking unit and/or milk line during machine milking.

Pulsation has a fundamental role in milk quality standard. Its use in machine milking aims to prevent teat edema and congestion and reduce the incidence of mammary infections, animal pain and discomfort during milking (Mein *et al*., 2003). The regulation UNI ISO 5707:2001 prescribes that phase "b" (milking) and phase "d" (massage) must last, respectively, at least 15% and 30% of the time required for each pulsation cycle for dairy cows. The latter value is a threshold under which a considerable increase in teat thickness occurs, thus favoring new udder infections (Hamann & Mein, 1996). Guidelines on the recommended minimum duration of each phase of machine milking of sheep and goats have not yet been developed. However, since the tissues of such species are more sensitive than those of cows, the results obtained with the latter species are very likely to be valid for small ruminants as well (Eitam & Hamann, 1993).

The milking unit is the component of the milking system that influences milking efficiency the most, in terms of udder emptying and vacuum stability. The design of all the elements that make up the milking unit (liners, claw, milk tubes) is aimed at facilitating milk flow from the mammary gland to the milk line and reducing to a minimum the vacuum fluctuations under the teat. Several studies have demonstrated that vacuum fluctuations are greatly associated with an increase in mastitis infection.

Most diseases of the mammary gland are caused by the use of improper liners, i.e., insufficient elasticity and inadequate diameter of the mouthpiece lip in relation to teat dimensions. If the diameter of the mouthpiece lip is too low, the base of the teat shows a purple ring due to irritation. Conversely, if such diameter is too high, the liner climbs up the mammary gland, thus slowing down or stopping the milk flow and exposing a larger surface of the teat to vacuum.

13.10.8 Stocking density

Stocking density has been shown to directly affect the levels of gaseous pollutants and airborne particles in animal houses. Differences in ambient levels of microorganisms depend largely on the amount of urine and feces produced per unit volume of bedding and on the pressure exerted by the animals. Both factors decrease the absorptive capacity of the litter and enhance decomposition and fermentation of the excrement. In addition, reduced space allowance has been related to social instability, greater stress and increased aggression, thus reducing immune competence of farm animals. In lactating animals, high stocking density has been recognized as having a deleterious effect on milk yield and to predispose to mastitis infection. A space allocation of 4.5–7.8 m^2 has been recommended for dairy cows, depending on body weight and breed (Blowey, 1994), and of 2 m^2 for dairy sheep (Sevi *et al*., 1999). High stocking density results in higher air concentrations of total microorganisms and coliform bacteria. As stocking density increases, the SCC rises and so does the concentration of mesophilic bacteria, psychrotrophs, and of total and fecal coliforms. The incidence of mastitis rises and infection appears earlier as the surface area per animal decreases.

13.10.9 Diseases

Disease conditions such as mastitis have a significant effect on both the yield and composition of milk, because they alter the permeability of the udder tissue and impair the ability of the secretory tissue to synthesize milk constituents. Mastitis destroys the secretory tissue in the udder, which in turn reduces milk production (Schmidt, 1971).

Fernando *et al*. (1982) reported that subclinical and clinical mastitis cause a rise in cow milk sodium chloride and a fall in potassium and lactose, with a net increase in electrical conductivity. Mastitis also results in an increase in the globulin level, a smaller increase in serum albumin and proteose content, and a decrease in casein content. In a study by Waite and Blackburn (1957), milk with SCC less than 100 000 cells/mL had no subclinical mastitis and no change in the chemical composition of the milk. As the SCC elevated from 100 000 to 500 000 cells/mL, there was a decrease in SNF and lactose content of the milk. When the SCC was above 1 000 000 cells/mL, the casein content began to decrease. However, mastitic conditions are not clearly correlated with SCC in goat milk, so that SCC may not represent changes in composition and yield of goat milk (Park & Humphrey, 1986; Park, 2010).

Poor hygiene of the air and of building surfaces is potentially a serious limitation to achieving high efficiencies of production and good health in intensive systems of animal husbandry. A relationship between the concentration of airborne microorganisms and the hygienic quality of milk and udder health has been demonstrated for dairy cows and sheep (Barkema *et al*., 1999; Sevi *et al*., 1999).

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13.10.10 Colostrum

The milk produced 1–5 days after parturition is called colostrum, and usually contains higher nutrient contents than normal mature milk including total solids, protein and ash. The most remarkable difference between colostrum and normal milk is the protein content, especially immunoglobulins. The newborn calves, lambs and kids can absorb immunoglobulins containing antibodies during their first day of life. After the first day, because of changes in the absorptive ability of the intestine, the digestive enzymes break down the globulins so that they lose their ability to provide immune protection of the neonate.

Colostrum contains 10 times more vitamin A than mature milk. Colostrum also has a higher content of calcium, magnesium, phosphorus, and chlorine, and is lower in potassium than normal milk (Schmidt, 1971; Park, 2010). Colostrum of cows and goats have a similar secretory pattern of nutrient composition.

13.10.11 Others

Although all the other factors listed in Table 13.7 have not been elaborated here, milk composition and yield can also be influenced by many factors, for example age at parturition, gestation and dry periods, body condition at parturition, and plane of nutrition. Increasing the energy intake increases the level of milk production to the animal's inherited potential (Park, 2010).

13.11 FACTORS AFFECTING QUALITY OF RAW MILK BEFORE AND AFTER MILKING

There are numerous factors involved in the production of quality raw milk such as Grade A and Grade B milk. Proper care of milking animals, as well as careful management of the dairy farm, milking and processing personnel, are essential prerequisites for the production of Grade A milk. Milk is subjected to rapid deterioration, since it is an excellent culture medium for bacteria and easily changed by environmental conditions such as light, temperature and oxygen level. Clean milking environments and milk handling before and after milking are especially important for the production of high-quality milk and dairy products.

13.11.1 Factors affecting quality of raw milk before and during milking

As summarized in Table 13.8, proper management that includes feeding a balanced diet, proper handling and cleaning of milking barns and milking parlor, and proper ventilation before and during milking are essential for the production of Grade A quality milk. Milking animals have to be maintained in a healthy and mastitis-free herd. Table 13.9 depicts the 5-point Mastitis Control Program for reduction of SCC as promoted by the National Mastitis Council, USA, and is widely recommended to allow the production of high-quality and low-SCC milk (Haenlein, 1993; Park, 2010).

Table 13.8. Factors affecting composition and quality of milk before and after milking.

Management and dietary factors
Proper feeding (i.e., balanced diet, amount of feeding)
Percent of roughage feeding
Proper handling of animals
Proper ventilation
Proper cleaning of barns and milking parlor
Before and during milking
Feeding and handling (i.e., objectionable odor) before and during milking
Use of recommended detergent, acid and sanitizers
Cleanliness of udder and teats, as well as of milking workers
Use of properly functioning and clean equipment
Handling of the milk during and after milking
Attentiveness of dairy plant workers
Post-milking and processing
Cooling
Transportation
Pasteurization
Processing
Packaging
Processing utensils
Post-pasteurization contamination

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Table 13.9. The 5-point Mastitis Control Program for reduction of somatic cell counts (SCC).

1. Use only functionally adequate milking machines, or hand milking in the correct manner
2. Dip teats after each milking with an effective, approved product
3. Administer promptly a full series of recommended treatments to all clinical cases of mastitis
4. Treat udder halves at drying-off of goats with an approved antibiotic preparation for drying-off
5. Cull animals with chronic infections when they do not respond to treatments

Courtesy of National Mastitis Council, USA (2010).

With regard to factors influencing the production of quality milk prior to and during milking, any objectionable odors should be prevented from contact with the milk, and udder, teats, milking equipment and milking personnel

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should be clean and hygienic. The milking workers should use the recommended detergent, acid and sanitizers, and should routinely check the cleanliness and functioning of the milking equipment and other auxiliary tools.

13.11.2 Factors affecting quality of raw milk after milking

Factors associated with maintaining the high quality of milk after milking are also important and are displayed in Table 13.8. These factors include, but are not limited to, cooling the raw milk, transportation of the raw milk to the milk processing facility, pasteurization and processing, packaging, handling the processing equipment and utensils, and post-pasteurization contamination (Park, 2010).

In order to maintain the high quality and superior flavor of milk after milking, the raw and pasteurized milks have to be carefully monitored to prevent off-flavor development by lipolysis. Three types of lipolysis can occur: (i) induced, (ii) spontaneous, and (iii) microbial. It has been reported (Deeth & FitzGerald, 1976; Park, 2010) that induced lipolysis can be caused by farm factors, processing factors, and dairy plant factors including transportation, agitation and foaming, homogenization, activation by temperature change, freezing and thawing, mixing, separation and poor refrigeration. Spontaneous lipolysis can occur via two main factors:

1. Milk processing factors, such as cooling, mixing, and separation, which disrupt milk fat globule membranes.
2. Animal factors such as stage of lactation, feed, season, breed, mastitis, milk and fat yield, and physiological factors.

In microbial lipolysis, many microorganisms contaminate dairy products, produce lipase, and cause the development of rancid flavor. The psychrotrophic bacteria are the most common sources of lipases. Bacterial lipases are different from milk lipases in that they are not inactivated by pasteurization and can attack the intact fat globules in milk (Deeth & FitzGerald, 1976; Park & Guo, 2006; Park, 2010).

13.12 PASTEURIZATION AND POST-PASTEURIZATION TREATMENTS FOR PRODUCTION OF QUALITY MILK

Removing harmful bacteria in milk is essential for food safety. Pasteurization is therefore the critical step for processing the raw milk. In addition, prevention of cross-contamination before, during and after pasteurization is extremely important for ensuring the safety of the processed milk products. In a cooperative effort between the FDA and IDFA, guidelines on pasteurization and post-pasteurization contamination were originally issued in September 1986, revised in 1987, and again revised by the IDFA in 1998. The following guidelines on pasteurization, vat pasteurization and post-pasteurization contamination have been issued by the FDA and IDFA.

13.12.1 Pasteurization

The basic pasteurization principle of the PMO code states that "every particle of milk or milk product be heated to at least a minimum temperature and held at that temperature for at least the specified time in properly designed and operated equipment." All dairy processing plants must assess the adequacy of their pasteurization equipment to determine whether or not they satisfy the basic principle of pasteurization. All processing plants must also recognize that dairy products with higher fat content and/or added sugars, or which are viscous (e.g., frozen dessert mixes, cream, eggnog), require a higher pasteurization temperature and/or longer times (International Dairy Foods Association, 1998). The standard time and temperature conditions for pasteurization of milk and high solids dairy products recognized by the US Public Health Service and the FDA are shown in Table 13.10 (Park, 2010).

The regulatory agencies also strictly enforce regulations that require a properly designed, installed, and operating flow diversion device and properly operating pressure controls for regenerator systems for all high-temperature short-time (HTST) pasteurization systems. It is also recommended that all Grade A products as well as frozen dessert mixes should be pasteurized in the final processing and packaging plant.

The PMO regulations are also enforced for pasteurization temperature and conditions in order to ensure the production of quality Grade A pasteurized milk. It is recommended that any product that has been mishandled, not protected from contamination, or which has not been maintained at a temperature of 7°C (45°F) or less should be discarded. External carton contamination with *Listeria* and *Yersinia* has occurred and may lead to product contamination. All milk and milk products are to be discarded if they have overflowed, leaked, been spilled, or improperly handled. When the handling and/or refrigeration of such milk and milk products is not in compliance with this requirement, they shall be discarded. Milk and milk products from damaged, punctured, or otherwise contaminated containers or product from out of code containers shall not be re-pasteurized for Grade A use (Park, 2010).

13.12.2 Vat pasteurization

The PMO codes are also enforced for all milk products processed by vat pasteurization to meet the basic requirements for pasteurization as defined by the regulations.

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Table 13.10. Minimum pasteurization temperature and times.*

Product	Temperature	Time	Reference method
Milk	145°F (62.8°C)	30 min	LTLT
	161°F (71.7°C)	15 s	STHT
	191°F (88°C)	1 s	UHT
	194°F (89°C)	0.5 s	
	201°F (94°C)	0.1 s	
	204°F (96°C)	0.05 s	
	212°F (100°C)	0.01 s	
Milk products of 10% fat or more or added sugar (half/half, cream, chocolate milk)	150°F (65°C)	30 min	
	166°F (74.5°C)	15 s	
	191°F (88°C)	1 s	
	194°F (89°C)	0.5 s	
	201°F (94°C)	0.1 s	
	204°F (96°C)	0.05 s	
	212°F (100°C)	0.01 s	
Eggnog and frozen dessert mixes	155°F (68.5°C)	30 min	
	175°F (79.5°C)	25 s	
	180°F (82.5°C)	15 s	

*Recognized by US Public Health Service and Food and Drug Administration. Reproduced from FDA data.

Proper pasteurization is critical for the production of quality pasteurized milk. In order to secure proper pasteurization, the following rules and regulations must be assured. Recording and indicating thermometers must be present and functioning properly. The need for a headspace heater functioning at 5° above minimum pasteurization temperatures is necessary to assure that any product that enters into the headspace is also properly pasteurized (International Dairy Foods Association, 1998; Park, 2010).

It is known that vat pasteurization systems can develop a variety of serious problems, such as lack of proper controls, leaking valves, improperly operated headspace heaters, and other serious defects. Controls must be accurate, valves and connections must not contain pockets of cold milk, foam (an excellent insulator) should be minimized in the vat during heating and holding, covers must remain in place during and following heating, and so on. The heat exchanger (presses) of HTST pasteurizer units need to be routinely opened and closely evaluated for stress cracks, pin holes, gasketing, and cleaning. Holes in regenerator and cooling plates can develop and cause contamination (International Dairy Foods Association, 1998; Park, 2010).

13.12.3 Post-pasteurization contamination

Contamination after pasteurization of milk and its products is a serious problem for food safety because of deterioration of and uncertainty about the pasteurized products. Commercial dairy processors and/or individual dairy operators should attempt to minimize the amount of handling, exposure to plant environment, and time/temperature abuse of the product after pasteurization (i.e., holding at elevated temperatures for extended periods of time). This aim can be accomplished by keeping the number of processing steps and storage time to a minimum after pasteurization (International Dairy Foods Association, 1998).

There are many sources of contamination after pasteurization of milk. One of the main sources of contamination for pasteurized dairy products results from contaminated sweetwater and leaking plates. It is recommended that the sweetwater and glycol of chill water systems be thoroughly checked. A scheduled review program should be initiated to assure that they are properly protected and do not contain harmful organisms. Any equipment, such as storage tanks and jacketed vessels, that utilize sweetwater or glycol solutions must be routinely monitored periodically for leaks and cracks (International Dairy Foods Association, 1998).

Proper sanitizers must be used at the appropriate strength and contact time. Cleaning and sanitizing regimens should be reviewed for proper times, temperatures, pressures, and flow rates. Review and assessment of the effectiveness of the cleaning and sanitizing regimen has to be practised. In addition, improper absorbent items, brushes, sponges, wooden tools, cracks and crevices in silo tanks, leaking valves, agitator shafts, shielding, and venting can all be sources of harborage and spreading of microorganisms in the plant environment. These areas should be carefully monitored on a scheduled basis. Impervious materials (i.e., plastic or metal) should be used to prevent bacterial growth and post-pasteurization contamination of the processed products (International Dairy Foods Association, 1998; Park, 2010).

Prevention of post-pasteurization contamination can also be achieved by protecting contamination of any product recovered from defoamer systems, by maintaining the temperature at or below 7°C (45°F) at all times, and by re-pasteurization. Imperfectly capped or filled containers/packages must be thoroughly checked. Manual handling/filling/capping of containers/packages should be eliminated, due to the occurrence of product contamination at filling/packaging operations. Mandrels, drip shields, bottom and top breakers, prefilling coding

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equipment, cutting blades, drain tables, box molders and brine tanks are critical areas where environmental contamination may occur. Also, constant monitoring is necessary for overhead shielding, conveyor belts, chain rollers, and lubricants to avoid possible contamination to the finished products (International Dairy Foods Association, 1998; Park, 2010).

The dairy processing manager/operator should review the adequacy of cleaning procedures for all processing and filling equipment, as well as piping. Potential areas of post-pasteurization contamination should be routinely checked and corrected. Environmental contamination of product and product contact surfaces should be minimized at all times. Additional care and shielding should be sought to minimize the post-pasteurization contamination of milk (International Dairy Foods Association, 1998; Park, 2010).

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14
Sanitary Procedures, Heat Treatments and Packaging

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14.1 INTRODUCTION

Heat treatment is a prerequisite for most types of milk processed for human consumption. The main purpose is consumer safety by inactivation of pathogenic microbes and their toxins in raw milk. Heat treatment of milk ensures a considerable storage period and shelf-life for the product by inactivating spoilage microorganisms and endogenous milk enzymes, depending to some extent on the processing conditions of time and temperature, whereas packaging type and storage conditions extend the heat treatment effects. The efficacy of processing is mainly affected by the handling of raw milk prior to heat treatment. Ideally, heat treatment aims to inactivate all microorganisms and their spores in raw milk. However, the applied time/temperature conditions and the means used for energy transfer into the milk, i.e. direct or indirect heating, affect the physicochemical, technological and nutritional characteristics of milk by promoting several mostly undesirable heat-induced reactions. Over recent years, research in the dairy industry has focused on alternative methods to the well-established heat treatments, for example ohmic heating, ultrasonication, microwave, high-intensity pulsed electric fields and high hydrostatic pressure. However, because of its efficacy and robustness and the fact that it is a continuous process, heat treatment remains the most convenient method for the sanitation of milk for human consumption.

The heat treatment of milk is a significant topic in dairy technology books and the subject of numerous important research papers. The main concerns with regard to the keeping and nutritional qualities of processed milk for human consumption, in addition to its chemical composition, are:

- the microorganisms in raw milk and relevant sanitary procedures;
- the conditions and type of heat treatment and packaging; and
- the heat-induced changes in the final product.

This chapter is divided into three sections that cover these concerns with regard to market (beverage) milk (i.e. mainly cow milk). Functional drinking milks, i.e. low lactose or fortified milks, are not included in this review.

14.2 SANITARY ASPECTS RELATED TO RAW MILK

14.2.1 Important microbiological aspects

Milk has superior nutritional and technological properties, but its drawback is that it is a rich substrate for the growth of a diverse microflora, including pathogens. Raw milk must come from animals without any symptoms of diseases that can cause illness to humans through consumption or

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handling of milk, and dairy herds must be officially free from tuberculosis and brucellosis. The microbiological status of raw milk determines its quality and its chemical composition. Counts and types of microorganisms influence the keeping quality of raw milk and the efficacy of heat treatments. The microbiology of raw milk has been the subject of many dairy textbooks (Varnam & Sutherland, 1996; Hayes & Boor, 2001; Chambers, 2002; Walstra *et al*., 2006; Jooste & Anelich, 2008), which have been consulted for this chapter. Because of the importance of the microbiological composition of raw milk, molecular techniques have been introduced that provide new information about new strain identification and microbial ecology in raw milk (Whiting *et al*., 2010; Quigley *et al*., 2011).

Milk delivered from the farm to processing plants contains many kinds of microorganisms in variable amounts depending on the health of the cows, their environment, feed, milking equipment, water supply, labour, cooling, and transport and storage conditions. Pathogenic and spoilage microorganisms are undesirable in raw milk. Pathogens entering milk will cause infection or food poisoning by forming toxins; these organisms are mainly bacteria but they can also be moulds producing mycotoxins, cysts of protozoans, or viruses. Spoilage microorganisms can grow rapidly in milk in contrast to pathogens. The initial microflora of raw milk results from contamination during milk production, collection and handling on the farm and during transport to the processing plant (Table 14.1). The microbiological status of raw milk intended for human consumption after processing is regulated by law. According to European legislation (European Union, 2006), raw cow milk must have a geometric average bacterial plate count at 30°C of 100 000/mL or less (over a period of 2 months with at least two samples a month). Immediately before processing into dairy products raw cow milk must have a plate count of 300 000/mL or less. Also, an upper limit has been set for somatic cell count (SCC) in cow milk in Europe, i.e. the geometric average must be 400 000/mL or less over a period of 3 months, with at least one sample per month. Cow milk in the USA must not exceed the SCC limit of 400 000/mL and 1 000 000/mL for goat milk (Grade A Pasteurized Milk Ordinance, 2009). Raw cow milk produced in Canada must have an aerobic mesophilic bacterial count of 50 000 colony-forming units (cfu)/mL or less and 1 000 000 SCC/mL or less (National Dairy Code, 2011). Raw milk for direct human consumption as drinking milk is not generally encouraged, because even the most appropriate hygienic procedures do not always ensure the absence of pathogens. The establishment of national rules that prohibit or restrict the marketing of raw milk for human consumption is allowed by the European Union (2004). In Standard 1.6.1 of Australia and New Zealand Food Standards Code (2011), microbiological criteria for unpasteurised milk for retail use are set for *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* and standard plate count.

The negative relationship of SCC with cow milk yield and the physicochemical characteristics of milk has been studied extensively. High SCC is correlated with enhanced activity of some endogenous proteolytic milk enzymes (e.g. plasmin, lysosomal proteinases) that can cause various changes in milk constituents and impair the quality and shelf-life of processed milk by inducing rancidity and bitterness because of higher levels of lipolysis and proteolysis (Ma *et al*., 2000). Barbano *et al*. (2006) concluded that at a bacterial count below 25 000 cfu/mL, raw milk SCC is the most important factor for the shelf-life of pasteurised milk with regard to the appearance of off-flavours. Also, age gelation in UHT milk produced from high SCC raw milk is connected with high levels of protease activity (Kelly & Foley, 1997; Datta & Deeth, 2001, 2003).

Among the various sources of bacterial contamination in milk, a mastitic udder and the milking equipment are the most serious, since they can increase the bacterial counts of raw milk by several million cfu per millilitre. Moreover, the winter housing of animals can substantially increase the bacterial count in milk. Although there are several antimicrobial systems in milk, i.e. polymorphonuclear neutrophils (PMNs), immunoglobulins, the lactoperoxidase–thiocyanate–H_2O_2 system and lactoferrin, spoilage microorganisms can grow easily and quickly because milk contains many kinds of nutrients and when the temperature is favourable. Spoilage microorganisms comprise mainly bacteria grouped as psychrophiles, mesophiles and thermophiles, which can grow optimally at 0–20, 20–45 and 45–60°C, respectively. The growth curve of bacteria has four distinct parts: the lag phase, exponential or log phase, stationary phase, and dying-off phase. The multiplication of bacteria is a geometric progression and their growth rate during the log phase is determined by the generation time (g), which is the time needed for a full cell division increase. Therefore, temperature is the crucial factor for the quality of raw milk and refrigeration is used as an effective means to preserve its quality. At 30°C, the generation time of the most important bacterial groups of milk is around 30 min, whereas at 5°C it ranges between 4 and more than 20 hours (Chambers, 2002; Walstra *et al*., 2006). Milk immediately after milking should be stored in cooling bulk tanks at 7°C or less on the farm, and then transported by cooling tank trucks to storage silos and held at 3–5°C until processing; deep cooling at 2°C is suggested. According to European Union legislation (European Union, 2004), the raw milk on the farm must be stored at 8°C or less immediately after milking or at 6°C or less if a daily collection scheme does not take place. During transportation to the processing units, the temperature must be kept below 10°C.

Table 14.1. Microbial contamination during milking and handling of raw milk.

Group	Sources/technological behaviour
Lactic acid bacteria: *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*	Widespread Contamination from soil, feed, milking and storage equipment Mesophilic: are killed by pasteurisation and for the most part by thermisation Thermophiles like *Streptococcus thermophilus* survive low pasteurisation
Pathogens: Salmonellae, *Staphylococcus aureus*, strains of *Escherichia coli*, *Mycobacterium tuberculosis*, *Campylobacter jejuni*, *Coxiella burnetii*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Leptospira interrogans*, *Bacillus anthracis*	Sources include mastitic udder, skin, hair, feet of the animal, faeces, milkers Killed by pasteurisation
Coliforms: Enterobacteriaceae, i.e. strains of *E. coli*, *Klebsiella aerogenes*, *Aerobacter*	Sources include biofilms on milking equipment, faeces, water, soil, milkers Killed by low pasteurisation Their existence in pasteurised milk is indicator of post contamination
Psychrotrophes (thrive at 3–7 °C): strains of *E. coli* *, *Y. enterocolitica**, *L. monocytogenes**, *Pseudomonas* sp., *Achromobacter*, *Aeromonas*, *Alcaligenes*, *Chromobacterium*, *Flavobacterium*, *Bacillus*, *Clostridium*, *Arthrobacter*, *Microbacterium*	Sources include milking equipment, cold chain at farm level, water *Pseudomonas* sp. is the most abundant in milk, especially *Pseudomonas fluorescens* They produce heat-stable lipases and proteinases
Thermoduric (i.e. can survive pasteurisation): *Microbacterium*, *Micrococcus*, *Bacillus* spores, *Clostridium* spores, *Alcaligenes*, thermophilic streptococci	Sources include milking equipment, feed, soil, water Spores are highest in winter, originating from bad silage and animal bedding Can grow in pasteurised milk Killed at 80°C for 20 s
Yeasts and moulds	Sources include air, feed, milking units and surfaces Low heat resistance

*Pathogens.

Sources: based on data from Varnam & Sutherland (1996), Sørhaug & Stepaniak (1997), Chambers (2002) and Walstra *et al.* (2006).

The microbiological quality of raw milk delivered to dairy processing plants depends on:

- the initial bacterial count, which should be low in milk from healthy animals (<10^3 cfu/mL) and comprises mainly streptococci and micrococci; however it can be as high as 10^6 cfu/mL if it is collected and handled under non-hygienic conditions (contaminated surfaces of milking equipment) and by improper cooling;
- the type of microorganisms, i.e., the presence of pathogens is generally not accepted and the initial psychrotrophic counts must be very low due to their potential for growth in raw milk kept for long periods under low temperatures;
- storage conditions, i.e., temperature, duration of storage and storage facilities, can substantially elevate the numbers and change the ratios between the various types of microbes.

14.2.2 Pathogenic microorganisms

With respect to pathogens, the following are of particular importance when designing heat treatments for milk (Varnam & Sutherland, 1996; Chambers, 2002; Walstra *et al*., 2006):

- *Mycobacterium tuberculosis* from infected animals or labour is the most heat-resistant non-spore-forming Gram-positive pathogenic bacterium in milk, but is killed by (low) pasteurisation, i.e., 72°C for 15 s.
- *Coxiella burnetii* (*Rickettsia* group), which may be carried by animals and which causes Q-fever, is killed by (low) pasteurisation.
- The Gram-positive spore-forming genera *Bacillus* and *Clostridium* of the Bacillaceae family are found mainly in soil, feed or water. *Clostridium perfringens* and

Clostridium botulinum produce toxins but they are strictly anaerobic. However, the aerobic or facultatively anaerobic *Bacillus cereus* is very important for dairy technology. It is a psychrophile and can produce food-poisoning enterotoxins when present at high numbers (~10^7 cfu/mL). However, in such cases milk is visibly degraded due to undesirable flavours known as sweet curdling and bitty cream.

- When high counts of some strains of *Staphylococcus aureus* are present in raw milk, a heat-stable toxin is produced. However, the growth of this organism is suppressed at low keeping temperatures.

Low-temperature pasteurisation is sufficient for the inactivation of the vegetative forms of pathogens that can contaminate milk. However, the psychrotrophic bacteria like *Pseudomonas* sp. that can grow at refrigeration temperatures and the thermoduric bacteria like *Micrococcus* sp. that can grow at about 50°C are very important for raw milk and its products. Although this group is of minor importance in raw milk due to their low counts, the opposite is true for pasteurised milk because of their resistance to pasteurisation conditions. Both groups influence the qualities of heat-treated and refrigerated stored milk. Also, bacteria of the genera *Bacillus* and *Clostridium* that can form endospores are dangerous. Spores may be released and activated in the milk after cell lysis induced by heat treatments and are a major concern for many milk processes.

14.2.3 Psychrotrophic microorganisms

Several reviews have summarised the significance for dairy processing of psychrotrophes that can grow at temperatures close to 0°C (Cromie, 1992; Champagne *et al*., 1994; Shah, 1994; Sørhaug & Stepaniak, 1997). The main psychrotrophic microflora of raw milk comprise Gram-negative rods, predominantly *Pseudomonas* sp. (>50%) (Champagne *et al*., 1994). Other genera include *Achromobacter*, *Aeromonas*, *Alcaligenes*, *Chromobacterium* and *Flavobacterium*. The Enterobacteriaceae, which includes coliform bacteria, accounts for 5–33% of the psychrotrophic microflora. Psychrotrophic Gram-positive bacteria (*Arthrobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Lactobacillus*, *Listeria*, *Microbacterium*, *Sarcina*, *Staphylococcus* and *Streptococcus*) are present in fewer numbers than the psychrotrophic Gram-negative rods. Many of these microorganisms produce extracellular proteinases and lipases that hydrolyse milk fat and proteins producing off-flavours such as rancidity and bitterness. Although psychrotrophs in general, and in particular *Pseudomonas* sp., are killed by low pasteurisation, their enzymes are heat-stable and can produce off-flavours later during the shelf-life of the product. Off-flavours can be evident when *Pseudomonas* spp. are present at greater than 10^6/mL in raw milk. Most extracellular proteinases from *Pseudomonas* are metalloenzymes containing one zinc atom and up to eight calcium atoms per molecule and have milk-clotting activity. They hydrolyse caseins but not whey proteins. Also, in addition to the very active extracellular lipase, the production of different phospholipases from psychrotrophs has been reported. Another problem related to psychrotrophs is the survival of the spores of *Bacillus cereus* after heat treatment, which can seriously affect pasteurised milk and cream. Surviving spores are activated by heat treatments in the range 65–75°C and the vegetative cells can grow at temperatures as low as 6°C. The pathogens *Listeria monocytogenes* (Gram-positive facultatively anaerobic rod) and *Yersinia enterocolitica* (Gram-negative facultatively anaerobe) are also psychrotrophs.

The proteolytic activity of psychrotrophs can result in coagulation of milk without causing acidification. Phychrotrophic counts around 7–8 log cfu/mL decrease shelf-life of pasteurised milk and can increase fouling in the heat exchanger, but 5.5 log cfu/mL can impair product flavour (Sørhaug & Stepaniak, 1997). Similar counts can cause gelation of UHT milk after 20 weeks of storage, whereas log counts around 7 can cause gelation after 2–10 weeks, lack of freshness and unclean/bitter flavours.

The production and accumulation of enzymes of psychrotrophs in milk can be avoided by using milk with low microbial counts, by decreasing the duration of refrigeration storage of raw milk or by applying a mild heat treatment (thermisation) to raw milk immediately after milking. Thermisation (i.e. heating at 57–68°C for >15 s and then cooling at <4°C) kills nearly all psychrotrophs and many lactic acid bacteria and extends the cold storage period of raw milk up to 4 days.

14.2.4 Non-microbial contaminants in milk

Apart from microbes other types of contaminants can cause hazards in raw milk but are not the objectives of this review. Such hazards include fungal metabolites like aflatoxin M1 associated with feeds, antimicrobials such as antibiotics, detergent and disinfectant residues, environmental and feed pollutants like dioxins, heavy metals and pesticide residues. Determination of the presence of aflatoxins and antibiotics is in fact included in the standard quality control requirements for raw milk intended for human consumption. There are numerous research papers and book chapters about the occurrence and detection of these compounds in milk (Walstra *et al*., 2006; Jooste & Anelich, 2008; Pradini *et al*., 2009; Nollet & Toldrá, 2010).

14.2.5 Handling of raw milk: measures for controlling its keeping quality prior to processing

14.2.5.1 Biofilm control

Low initial microbial counts, clean and healthy animals, safe fodder and water supply, cleaning and disinfection of milking equipment, and rapid cooling are prerequisites for the quality of raw milk destined for processing. A very important issue associated with the quality of both raw and processed milk is the development of biofilms on milk handling equipment, such as milking and storage units. Bacterial biofilms are surface-associated three-dimensional groups of bacterial colonies held together by glycocalyx produced during bacterial metabolism, and this can survive the cleaning process. Psychrotrophic *Pseudomonas* sp., *E. coli* strains, heat-resistant streptococci and *Bacillus* spores are involved in biofilms on milk handling equipment. Biofilm formation is favoured by the deposition of proteins and sugars from milk (Kulozik, 2002). Cleaning in place (CIP) protocols enabling intense pre-rinsing with water, circulation of alkali/acidic and sanitising solutions and final rinsing with water are required for milking equipment and storage units of raw milk (Chambers, 2002; Walstra *et al*., 2006).

14.2.5.2 Cooling and thermisation

Several approaches enable control of the microbial quality of bulk raw milk during its storage. The simplest is to lower the storage temperature; a decrease from 6 to 2°C causes substantial retardation of the growth of psychrotrophs up to the critical value of 10^6 cfu/mL for 3 days (Griffiths *et al*., 1987). As mentioned in section 14.2.3, to avoid the undesirable growth of psychrotrophs, which can be in excess of 5×10^5 cfu/mL after 4 days of refrigerated storage, a moderate heat treatment (57–68°C for >15 s) called thermisation followed by cooling at 4°C is applied. Thermisation kills nearly all psychrotrophs and many lactic acid bacteria, extending the cold storage period of raw milk to 4 days before processing. However, during the cold storage period endogenous milk enzymes are generally active. This is especially true for lipoprotein lipase, which can hydrolyse the triglycerides of fat globules when the milk fat globule membrane (MFGM) is damaged by temperature fluctuations or by agitation and foaming. The induced lipolysis in milk results in rancidity. Actually, lipoprotein lipase activity is only a problem in raw milk, because it is almost totally inactivated by pasteurisation. Its potential activity in raw milk is enough to produce rancid flavours in less than 10 min. Nevertheless, the activity of the enzyme decreases slowly in raw milk kept under refrigeration and provided that the milk triglycerides are protected by an intact MFGM, its action is controlled (Olivecrona *et al*., 2003; Walstra *et al*., 2006).

14.2.5.3 Lactoperoxidase system

Another approach for improving the keeping quality of raw milk is activation of the lactoperoxidase (LPO) system in milk. LPO is the predominant enzyme responsible for the antimicrobial properties of bovine milk, being active against bacteria, fungi and viruses in the presence of sufficient concentrations of hydrogen peroxide (H_2O_2) and thiocyanate ions (SCN^-). It is the second most abundant enzyme in bovine milk after xanthine oxidoreductase and it catalyses the oxidation of SCN^- by H_2O_2 to $OSCN^-$. Both SCN^- and $OSCN^-$ are harmless to the animal but inhibit most bacteria that produce H_2O_2 themselves. The activity of the LPO system in milk, which includes H_2O_2 (coming mainly from bacterial metabolism) and thiocyanate anions from cyanoglucosides in animal feeds, depends on SCN^- concentration and pH. Activation of the LPO system in milk through the addition of thiocyanate ions and H_2O_2 or addition of an H_2O_2 system (e.g. glucose oxidase) can be used for the preservation of raw milk during storage or for the extension of milk shelf-life, if applied prior to pasteurisation. In the absence of refrigeration or heat treatment, this treatment is known as 'cold pasteurisation' (Björck, 1994; Pruitt, 2003; Seifu *et al*., 2005).

14.2.5.4 Carbon dioxide addition

Carbon dioxide can be added to raw milk as an anti-microbial agent (Hendricks & Hotchkiss, 1997; Martin *et al*., 2003). Addition of 20–30 mmol/L at refrigeration temperatures has been proposed for the extension of storage of raw milk, while considering the pressure and temperature conditions of the treatment since these can cause precipitation of proteins (Rajagopal *et al*., 2005). The effect of CO_2 on various species and strains is variable. In general, the lag phase of aerobic plate counts increases substantially and more dramatically than the increase in psychrotrophs lag phase. Inhibition of coliforms by at least 1 log cfu/mL is also possible. This treatment has a more pronounced effect on Gram-negative bacteria than on Gram-positive bacteria and spores (Martin *et al*., 2003; Singh, P. *et al*., 2011). However, the reduction in pH caused by CO_2 may cause dissociation of the casein micelles, which can be detrimental for the efficacy of heat exchangers due to fouling (Loss & Hotchkiss, 2003).

14.2.5.5 Centrifugation, clarification and bactofugation

Physical treatments like centrifugation (clarification) are also employed to improve the keeping and technological quality of raw milk. The simplest approach is disc bowl separation, which can remove dirt particles, animal hair, somatic cells, and microorganisms and their spores in the form of sediment (sludge) due to the density difference

between them and milk serum. However, due to the inclusion of somatic cells and bacteria in the cold agglutinated fat globules, a large portion of these particles is in the cream phase especially when a fat separator is used at above 40°C, but can be removed with a modified centrifugal separator (clarifier). Moreover, high-speed entrifugation called bactofugation can be used to remove up to 99% of spores. Bactofugation removes spores but causes losses of about 3% of milk constituents. The centrifugate, also rich in caseins, is sterilised at 130°C for a few seconds and is then added to the milk. Bactofugation takes place at 60–65°C and removes up to 95% of spores in a single run. It is used for the extention of shelf-life of pasteurised milk; however, it is an expensive method and requires two separators in a row for the removal of 99% of spores (Spreer, 1998; Walstra *et al*., 2006).

14.2.5.6 Microfiltration

Filtration techniques such as microfiltration (pore size ~1 μm) operating at low pressure are also used to remove spores, microorganisms and somatic cells from raw milk prior to heat treatment. These milk constituents have diameters above 1 μm, similar to some of the fat globules, and therefore this process is applied to skimmed milk. The retentate and the cream are heat treated/sterilised independently before they are mixed with the permeate, the latter containing 0.1–1% of the initial counts (Elwell & Barbano, 2006; Walstra *et al*., 2006; Pouliot, 2008; Goulas & Grandison, 2008).

14.3 STRATEGIES FOR PRODUCING HEAT-TREATED MILK FOR HUMAN CONSUMPTION

Two principal terms are used to describe the treatment of market milk: pasteurisation and UHT (ultra high temperature). According to the relevant Codex Alimentarius (1993, 2004):

> *Pasteurisation is a microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. Pasteurisation conditions are designed to effectively destroy the organism* Mycobacterium tuberculosis *and* Coxiella burnetti.

whereas

> *UHT treatment of milk is the application of heat to a continuously flowing product using such high temperature conditions for such time that renders the product commercially sterile at the time of processing. When the UHT treatment is combined with aseptic packaging, it results in a commercially sterile product.*

The categories of milk produced for human consumption according to the applied heat treatment are presented in Table 14.2.

14.3.1 Pasteurisation

High-temperature short-time (HTST) pasteurisation is the minimum heat treatment applied to milk for human consumption that ensures consumer safety. The most common conditions are 75°C for 20 s, often referred to as low pasteurisation. Low-temperature long-time (LTLT) pasteurisation (63°C for 30 min or 68°C for 10 min) is not used in the industrial production of drinking milk since it is a batch procedure. Under these conditions milk has negative reaction to alkaline phosphatase (EC 3.1.3.1) and positive reaction to peroxidase (EC 1.11.1.7). Equivalent temperature–time combinations (°C/s) for pasteurisation are given in the Grade A Pasteurized Milk Ordinance (2009) and in the Australia New Zealand Food Standard Code (2011), i.e. 69.4/60, 72/15, 79/2, 89/1, 96/0.05, 100/0.01.

The factors that influence milk pasteurisation, from the quality of the raw milk to the storage conditions, have been reviewed by Boor and Murphy (2002) and Lewis (2003). Mixing with raw milk in the regeneration or cooling sections as well as non-hygienic conditions in storage tanks before and during processing are of particular interest. Accuracy of temperature and time, leakages and pressure must be constantly checked throughout processing. In this respect, there are legislation standards about coliform counts in pasteurised milk, which are indicative of poor hygiene or possible recontamination of heat-treated product. In two of five samples of pasteurised milk, Enterobacteriaceae counts must be below 5 cfu/mL (European Union, 2005). Bulk-shipped heat-treated products produced in the USA must have bacterial counts below 20 000/mL and coliform counts below 10/mL (Grade A Pasteurized Milk Ordinance, 2009). Furthermore, the storage temperature of pasteurised milk is very significant due to doubling of microbial spoilage for every 2°C increase, especially at temperatures above 6°C (Simon & Hansen, 2001; Rysstad & Kolstad, 2006).

Higher than normal milk pasteurisation temperatures, i.e. up to 90°C, have been studied with two objectives: (i) increased shelf-life and keeping quality of pasteurised milk and (ii) complete elimination of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), if present. MAP causes Johne's disease in ruminants and has been reported as a factor involved in Crohn's disease in humans. MAP can contaminate milk by direct shedding from infected cows, by faecal contamination or by mixing raw or pasteurised milk in the tanks or in the pasteuriser, respectively (Eltholth *et al*., 2009). As reported by Lewis (2003) the HTST pasteurisation of milk should be 72°C for 25 s to be efficient against MAP. The best keeping quality for pasteurised milk is achieved using temperatures below 77°C, which do not inactivate LPO and which do not activate the growth of spores by heat shock (Lewis,

Table 14.2. Heat treatments applied to raw milk* for human consumption.

Shelf-life and storage conditions	Aim	Heating conditions
(Low) HTST pasteurisation: refrigerated conditions at <7°C for 3–21 days depending on raw milk quality, processing and packaging conditions and the cold chain; usually 5–7 days	Inactivation of pathogens, moulds, yeasts and most bacteria	HTST at least 72°C for 15 s, usually 75°C for 20 s, followed by immediate cooling to ≤6°C
High-temperature pasteurisation: refrigerated conditions for 45–60 days	All vegetative microorganisms and most enzymes are inactivated	≥85°C for 20 s, usually 115–120°C for 2–5 s
UHT (ultra high temperature) treatment (always continuous operation): non-refrigerated conditions <32.2°C for 3–12 months	Absence of viable microorganisms and their spores Minimal chemical, physical and organoleptic changes or at acceptable levels Inactivation of almost all enzymes	135–150°C for 1–4 s; mostly at >140°C for 5 s, followed by 'flash cooling' in vacuum vessel
Sterilisation (batch or continuous in-container process): non-refrigerated conditions for 8–12 months	Inactivation of all microorganisms including spores and all enzymes	110–120°C for 20–40 min; two-stage process combining UHT at 135°C for 5 s, cooling to 40°C, filling into bottles and sterilisation at 117–123°C for 10–12 min, cooling to 20°C
ESL (extended shelf-life) technology†: 15–45 days at <7°C; ≥45 days at 10 °C for infusion heating	All vegetative microorganisms and most enzymes are inactivated; most spores removed	140 °C for 2 s by direct heating; combination of bactofugation, microfiltration and pasteurisation; 130–145°C for <1 s by means of direct infusion heating

*Raw milk intended for the production of heat-treated milk may be thermised (57–68°C for >15 s; positive reaction to phosphatase test) or initially heat treated in another establishment under time–temperature conditions lower than pasteurisation.

†Ultra-pasteurised milk produced in the USA and Canada (direct infusion heating at ~138°C for 2 s).

Sources: based on data from Codex Alimentarius (1993, 2004), Tetra Pak (1995), Varnam & Sutherland (1996), Henyon (1999), Boor & Murphy (2002), Lewis (2003), European Union (2004, 2006), Rysstad & Kolstad (2006), Walstra *et al*. (2006), de Jong (2008) and Lorenzen *et al*. (2011).

2003; Martin *et al*., 2003; Gandy *et al*., 2008). However, Simon and Hansen (2001) did not observe spore activation in their heat-shock experiments at 84 and 92°C. In addition to (low) HTST pasteurisation, the tem 'high-temperature pasteurisation' is also used (Table 14.2). This treatment results in negative phosphatase and peroxidase reactions and, in conjunction with ultra-clean or aseptic filling and packaging, results in a product with extended shelf-life.

14.3.2 UHT treatment

UHT milk is a microbiologically stable product characterised by a cooked flavour and long keeping times at room temperature (Table 14.2). However, it is not totally stable during storage. Proteolysis-induced defects like bitter taste, increase in viscosity or even sweet curdling can appear. The main factors responsible for these phenomena are plasmin and thermoduric bacterial enzymes produced before heat treatment and not totally inactivated by UHT treatment. During storage, vitamin loss and development of Maillard reactions has also been observed (Datta & Deeth, 2003; Elliott *et al*., 2005). A microbiological hazard associated with UHT milk is the recently identified, mesophilic, highly heat-resistant, non-pathogenic spore-former *Bacillus sporothermodurans*, which has been isolated from industrially UHT and sterilised milk. These types of microorganisms are known as heat-resistant spore-formers (International Dairy Federation, 2000). The pathway of contamination is not well understood, although it has also

been isolated from raw milk. Contamination of raw milk at the farm level through feed and milking equipment is considered the most possible route.

14.3.3 Extended shelf-life technology

Extended shelf-life (ESL) technology aims to produce drinking milk with prolonged storage and flavour close to pasteurised 'fresh' milk, in contrast to UHT milk, in which sensory and nutritional qualities have been impaired due to the high heat load (Table 14.2). Rysstad and Kolstad (2006) provide the following definition for this category: 'ESL products are products that have been treated in a manner to reduce the microbial count beyond normal pasteurisation, packaged under extreme hygienic conditions and which have a prolonged shelf life under refrigerated conditions'. According to de Jong (2008), ESL is a complete systems approach, being a combination of processing and packaging systems and distribution with respect to the key components of chilled liquid milk. Additional technologies like bactofugation can be introduced to remove bacterial spores. The aim of this process is the production of milk with organoleptic and nutritional characteristics close to those of pasteurised milk. There is not any legal definition for this category. Another approach is utilisation of microfiltration in conjunction with HTST (low) pasteurisation for the production of high-quality ESL milk (Elwell & Barbano, 2006; Hoffmann *et al*., 2006). High-temperature pasteurised and ultra-pasteurised milk produced in the USA and Canada can also be considered ESL milks.

14.3.4 Types of heat treatment

The kinetic parameters associated with the heat treatments of milk, along with factors that cause deviations, are presented in Table 14.3. Walstra *et al*. (2006) discuss some factors that impair the accuracy of the equations during heat treatment of milk. In brief, the complex composition of milk favours several consecutive reactions that may be of different order or of markedly different temperature dependence. Changes in the physicochemical conditions (pH, Eh) during heating must be considered. The *Z*-value is not constant within the wide range of temperatures used, resulting in curved log t'–temperature plots and the temperature profile. Also the type of heating complicates the mathematical description of milk heat treatments. Finally, the fact that some bacteria occur in clumps or dirt aggregates, which alters the heating performance, and the induction of spore germination by moderate heat treatment must be considered.

Fouling and biofilm formation on the walls of heating equipment are crucial for the efficacy of heating conditions and the selection of heating type. Fouling is the formation of heat-induced deposits: for temperatures up to 110°C these are relatively soft, consisting mainly of proteins (up to 70%) and is consequently named protein fouling. Above

Table 14.3. Kinetic parameters related to milk heat treatment.

Aspect	Description
Changes of milk constituents (e.g. protein denaturation) and enzyme inactivation	First-order kinetics: $-dc/dt = kc$ or $c = c_0 e^{-}kt$
Expression of heating duration	*D*-value (min) is the necessary time for the decimal reduction of the initial concentration of a milk constituent or bacterial counts
Formation of new substances due to heat treatment (e.g. products of Maillard reaction)	Zero-order kinetics: $c = kt$
Activation energy required to start a chemical reaction	E_a (kJ/mol) depends on the temperature according to Arrhenius equation: $k(T) = k_0 \exp(-E_a/RT)$
Expression of temperature dependence, i.e. of the *D*-value	*Z*-value, the increase in temperature necessary for a 10-fold increase in *D*
Expression of temperature dependence of a reaction	Q_{10}, the increase in rate constant resulting from increase in temperature by 10 °C
Presentation of adequate heating conditions	Linear plots of log t' against temperature in °C
Description of bacteria thermal death curves*	Weibull equation: $N = N_0 \exp-(t/a)^b$, $b > 0$
Sterilising effect	$\log N_0 - \log N$; $\log(N_0/N)$ = heating time/*D*

*Variable heat resistance and ability to stress adaptation of bacteria is not in accordance with first-order kinetics (Walstra *et al*., 2006).

c_0, initial concentration; k, reaction rate coefficient or rate constant; k_0, k when E_a ~0; N, final population; N_0, initial counts; R, gas constant; T, absolute temperature; t', duration of heat treatment necessary to cause a specific effect; t, duration of processing.

Sources: based on data from Clayes *et al*. (2002), Lewis (2003), Walstra *et al*. (2006) and de Jong (2008).

Table 14.4. Heating types utilised in the processing of milk for human consumption.

Type	Heat transfer	Cooling	Process
Indirect*	Plate heat exchangers, tubular heat exchangers and external holding tube	In the regeneration section of the heat exchanger unit	Pasteurisation, high pasteurisation, pre-heating of UHT milk
Direct injection†	Immediate condensation of injected steam on the milk moving as a pressurised stream	Vacuum expansion vessel for very rapid cooling and removal of the injected steam	High and ultra pasteurisation, UHT process
Direct infusion†‡	Immediate condensation of steam on milk sprayed in a vessel containing pressurised steam	Similar to direct infusion system	High and ultra pasteurisation, UHT process

*Not the best choice for high-, ultra-pasteurised and UHT milk due to the enhancement of fouling at high temperatures.
†Very high heating capacities.
‡The best choice for the production of milk with properties similar to pasteurised.
Sources: based on data from Tetra Pak (1995) and de Jong (2008).

this temperature the deposits are in the form of mineral fouling, which is hard and consists mainly of minerals (up to 80%) and which is due to the heat-induced insolubility of calcium phosphate and citrates (de Jong, 2008). The features of biofilms that can re-contaminate milk during the heating process are presented in section 14.2.5.1. Preheating at 80–95°C for 30–60 s is an essential step in UHT processing in order to avoid fouling-related problems, because under these conditions β-lactoglobulin is denatured for the most part, interacting with casein micelles by forming β/κ complexes. As a result it does not contribute to fouling on the surfaces of UHT processing equipment (Datta & Deeth, 2001). The temperature–time combination required for the various categories of milk for human consumption is the main criterion for the selection of a direct or indirect heating system, since high temperature-induced fouling can reduce substantially heat transfer through the walls of an indirect system. The types of heating utilised in drinking milk production are presented in Table 14.4.

In general, all types of heat processing are divided into four distinct phases: preheating, heating, holding and cooling (Tetra Pak, 1995; de Jong, 2008). In indirect systems, the heating medium and the milk are not mixed. This is the case for plate and tubular heat exchangers. In direct systems, milk is mixed with steam either by injection of the steam into the milk or by infusion of the milk into pressurised steam followed by cooling in a vacuum chamber that results also in the evaporation of the water condensed from the steam. As stated in Grade A Pasteurized Milk Ordinance (2009), with a 66°C increase with steam injection, there is a volume increase of 12% in the holding tube that must be considered in the calculations. Furthermore, in the same ordinance it is indicated that complete condensation of the steam inside the injector is ensured if there is no pressure fluctuations in the injection chamber and if steam is de-aerated in order not to add non-condensable gases to the milk.

A heating method must be efficient with regard to safety and the shelf-life of milk without changing the organoleptic properties of milk, considering (low) pasteurised milk as a reference. The direct infusion method is the most appropriate taking into consideration these prerequisites (Henyon, 1999). According to Rysstad and Kolstad (2006), infusion systems result in low chemical degradation due to the combination of accurate temperature and very short accurate heating times and minimal contact between product and surface, thus avoiding fouling. Processing lines and various strategies for the production of safe and acceptable milk for human consumption are discussed extensively by Tetra Pak (1995), Varnam and Sutherland (1996), Spreer (1998), Smit (2003) and Walstra *et al*. (2006).

Recently, a direct infusion process (72–120°C for 0.1–0.7 s) called instant infusion pasteurisation has been studied in comparison to HTST heating (Hougaard *et al*., 2009; Hammershøj *et al*., 2010). According to the results the inactivation of microorganisms in skimmed milk was at least the same as for HTST at 72°C for 15 s, but partial disruption of milk fat globules was observed. It was concluded that this technology is less gentle than pasteurisation but gentler than treatment at 85°C for 30 s. De Jong (2008) also presents the innovative steam injection system, a combination of extremely high temperatures (150–200°C) for extremely short time (0.1 s). This process dramatically reduces the thermophilic spores of *Bacillus*

stearothermophilus (>6 log) and *B. sporothermodurans* (3–4 log) and at the same time has a mild effect on whey protein denaturation (<25%). If a preheating step is included, bitterness associated with plasmin is not observed and the shelf-life at 7°C increases up to 60 days.

14.3.5 Packaging

Packaging is the barrier between the milk and the environment and must not allow the exit of food material or loss of its constituents while preventing entrance of microorganisms or chemical compounds from the environment or the packaging material into the milk. Moreover, and this is especially true for milk, it must offer protection against light and oxygen. The type and strategy of packaging is a determining factor for the shelf-life and keeping quality of milk and it is strongly connected with the heat treatments applied to milk (Table 14.2). Heat-treated milk can be packaged in glass bottles or single-use containers made from various packaging materials (Table 14.5). The transparency of packaging and its permeability to oxygen are very significant, since they can be the cause of oxidative degradation of riboflavin and the appearance of off-flavours. Poulsen (1995) grouped the light-induced changes in milk as follows: partial or total destruction of particular vitamins, acceleration of the development of oxidised flavours, and the development of 'activated' flavours, i.e. burnt or cabbagey. The complex effects of oxygen and light on milk vitamins is discussed in section 14.4.1.3.

Single-use plastic bottles preformed on site from polyethylene, polypropylene or polyethylene terephthalate (PET) can be used. Cardboard coated with polyethylene that can also incorporate aluminium foil, fabricated from roll material directly before filling, known as laminated foils, offer superior oxygen and light protection. Final sealing of bottles with an aluminium foil cap and heat sealing of the other containers are carried out. Sterilised milk is heated and sterilised in hermetically sealed containers, the seal of which must remain intact (Poulsen, 1995; Varnam & Sutherland, 1996; Rysstad & Kolstad, 2006; Walstra *et al*., 2006).

Aseptic packaging, which is part of UHT and recently of ESL technologies, assumes aseptic filling in a sterilised environment. Aseptic packaging into sterilised containers followed by hermetic sealing with a sterilised closure is applied. Filling is carried out within an aseptic zone bounded by structural features or sterile airflows, also including aseptic holding tanks. The package is sterilised either inside the packaging machine or externally and aseptically introduced into the aseptic zone (Codex Alimentarius, 1993; Tetra Pak, 1995). The aseptic zone may be sterilised utilising superheated steam or H_2O_2 or other physical or chemical agents or a combination. The process of sterilisation of the package must offer a rapid antimicrobial effect, be compatible with the material, be easily removed and be safe for the consumer. Most commonly, H_2O_2 combined with heat is used for plastic containers and laminated foils, i.e. rinsing with hot concentrated H_2O_2 (20–35%), which is easily removed by hot air (>100°C) providing an additional sterilising effect (Henyon, 1999; Rysstad & Kolstad, 2006; Walstra *et al*., 2006; Chavan *et al*., 2011).

Several research studies have shown that reduction in light-sensitive vitamins occurs starting with the photo-oxidation of riboflavin in translucent packages that are light and gas permeable, in addition to the formation and accumulation of volatile compounds that decrease the sensory and nutritional properties of market milk. The comparative study of Simon and Hansen (2001) on the use of various packaging materials for milk pasteurised at 76.4–92.2°C for 25 s showed that milk packaged in barrier

Table 14.5. Packaging materials often used for liquid milk for human consumption.

Material	Barrier to light	Barrier to oxygen	Resistance to autoclaving
Glass	No	Complete	Yes
Aluminium	Complete	Complete	Yes
Polycarbonate	No	High	Yes
Low-density polyethylene (LDPE)*	Very weak	Moderate	No
High-density polyethylene (HDPE)*†	Very weak	High	No
Polypropylene (PP)*‡	No	Rather high	Yes
Crystalline polyethylene terephthalate (CPET)*	No	Almost complete	Yes

* Pigment can be added to colour plastic packaging materials, e.g. TiO_2 for white colour.

† Appropriate for bottles made by means of blow moulding.

‡ Lids of the containers may be from LDPE.

Sources: based on data from Bosset *et al*. (1995) and Walstra *et al*. (2006).

and foiled boards did not deteriorate fast. According to the findings of Smet *et al*. (2009), when high-density polyethylene is used for UHT milk stored under illuminated conditions, hydrophilic antioxidants are consumed during the first days, whereas α-tocopherol content also decreases but at a later stage; lipid and oxidation products are formed when all these antioxidants are consumed. However, with a light barrier in the packaging, no oxidation products are detected throughout 3 months of storage. Papachristou *et al*. (2006) report that clear PET packaging with an ultraviolet (UV) blocker provided at least the same protection to pasteurised milk as paperboard cartons. Based on their spectral transmission curves, they suggest that incorporation of a UV blocking agent in combination with a dark coloured pigmentation like blue or green in pasteurised milk packaging provide better protection for light-sensitive vitamins, when shelf-life exceeds 5–6 days. White combined with yellow pigmented PET bottles are considered similar to brown glass bottles, with 5% or less transmittance at 450 nm (Saffert *et al*., 2006). According to Rysstad and Kolstad (2006), the best protection is provided by aluminium foil paperboard (0% transmission to light). According to Poulsen (1995), the maximum permissible light transmission for a milk packaging material should be 8% at 500 nm and not more than 2% at 400 nm. The package protection also depends on the type of heat-treated milk. Even though highly pigmented PET bottles with light transmittance below 10% at 450 nm protect pasteurised milk reasonably sufficiently within the limits of the usual shelf-life, light-induced changes cannot be avoided under usual retail storage conditions and this type of packaging cannot protect UHT milk effectively (Saffert *et al*., 2006, 2008). As reported in section 14.4.1.3 on vitamins, the lower redox potential of sterilised or UHT milk decreases its photosensitivity in comparison to low pasteurised milk; however, flavour defects related to oxygen effects can be observed. Incorporation of an oxygen scavenging film into the package (active packaging) has been used as a means for controlling the 'stale' flavour defect of UHT milk attributed to methyl ketones and aldehydes from lipid oxidation (Perkins *et al*., 2007). Significant reduction in dissolved oxygen levels and related individual volatiles was observed, but sensory evaluation results based on flavour were not affected by this treatment. Another strategy for heat-treated milk packaging, used in order to extend the shelf-life, is addition of carbon dioxide to counteract spoilage microorganisms (Singh, P. *et al*., 2011). Finally, environmental issues in terms of energy use, waste prevention, recycling or environmentally friendly materials are taken into consideration in the design of milk packaging (International Dairy Federation, 2000; Singh, J. *et al*., 2011).

14.4 EFFECTS OF HEAT TREATMENTS ON MILK

Heat-induced changes involve the major and minor constituents of milk as well as its physicochemical characteristics and technological behaviour. The complex composition of milk and the physicochemical equilibrium along with the diverse heat treatments applied to milk complicate the presentation of these changes. Two major types of effects occur (Elliott *et al*., 2003):

1. The effect on milk components of special interest for keeping quality and human nutrition. These changes mainly impair the quality of heat-treated milk and include degradation of lactose to organic acids and formation of lactulose, denaturation of whey proteins, destruction of some vitamins and enzymes, hydrolysis of proteins and lipids, and disturbance of calcium/phosphorus equilibrium.
2. The formation of new substances resulting mainly from Maillard reactions that result in cooked flavour and loss of nutritional value. Maillard reactions continue during the storage of high-heated milks.

According to the overview of kinetic constants for reactions in milk presented by de Jong (2008), the components of milk that can be affected by heat treatment present variable sensitivities in terms of k_0 and E_a (Table 14.3). For example, with regard to the destruction of microorganisms and spores in milk in the temperature range 60–140°C, ln k_0 ranges from about 57 to 174 and E_a from about 180 to 509 kJ/mol. However, lipases from psychrotrophic *Pseudomonas* require temperatures up to 150°C (ln k_0 ~22, E_a ~90 kJ/mol). Heat-labile constituents like endogenous enzymes are inactivated in the range 50–150°C, whereas wide ranges in ln k_0 (15–225) and E_a (64–663 kJ/mol) are observed.

O'Connel and Fox (2002) consider the following as the main heat-induced changes, which at temperatures above 100°C become irreversible:

- aggregation of micelles and dissociation of caseins from micelles because colloidal calcium phosphate links and hydrophobic bonds are weakened;
- thermal dephosphorylation and proteolysis of caseins;
- acidification due to thermal oxidation of lactose to organic acids, to the precipitation of primary and secondary phosphates with concomitant release of H^+, and to the dephosphorylation of casein followed by precipitation of the liberated phosphate;
- changes in micellar hydration and zeta potential due to acidification;

- Maillard reaction, in which mainly the ε-amino group of lysine and the carbonyl group of lactose are involved; and
- covalent polymerisation of proteins.

For changes caused in market milks at temperatures higher than pasteurisation (Walstra *et al*., 2006), the irreversible or slowly reversible reactions are the most important.

14.4.1 Effect on milk constituents

14.4.1.1 Proteins

Caseins are not heat labile and very severe treatment is necessary to dephosphorylate, aggregate or hydrolyse them. Whey proteins are characterised by high levels of secondary and tertiary structure and for this reason they are susceptible to heat denaturation that decreases their solubility and biological value. Their heat tolerance is as follows: α-lactalbumin > β-lactoglobulin > bovine serum albumin > immunoglobulins. The effect of heating on the heat-sensitive whey proteins has been studied extensively, since it is associated with:

- β-lactoglobulin/κ-casein complex formation, known as β/κ complex, which impairs rennetability of milk and syneresis of curds and initiates gelation in UHT milk;
- the heat-induced unfolding of β-lactoglobulin, which is involved in the denaturation of the very heat-tolerant plasmin;
- the residual β-lactoglobulin, and partly also α-lactalbumin, which serve as an index for the assessment of heat treatments of low intensities below the UHT level;
- the possible reduction of allergenicity of whey proteins and especially of β-lactoglobulin caused by heat treatments;
- the modification of their functional properties that are important for nutrition and health.

In particular, β-lactoglobulin but also α-lactalbumin are very significant in relation to their heat denaturation, considering that they are the most abundant whey proteins. At temperatures above 65°C, unfolding of β-lactoglobulin occurs, i.e. first stage of denaturation. Kinetics are first order: *D*-value at 75°C is 49.9 min and at 90°C is 3.5 min. Cystine disulphide bonds disrupt and along with the free sulphydryl group of the native molecule are available to participate in thiol–disulphide exchanges; they react intramolecularly or intermolecularly with κ-casein on the micelle surface mainly and with proteins on MFGM (Datta & Deeth, 2001; Clayes *et al*., 2002; Considine *et al*., 2007; Donato & Guyomarc'h, 2009). Therefore, β/κ complexes are a characteristic feature of all heat-treated milks. They are present not only on the casein micelle but also in the milk serum, apparently due to dissociation of caseins from the micelles. The β/κ complex formation is the first stage in the age gelation of UHT milk, which impairs its keeping quality. The second stage of this phenomenon takes place after the release of this complex from the casein micelles by the action of plasmin or bacterial proteinases, which are heat stable, i.e. from psychrotrophs. The liberated complexes can cross-link to form a protein gel in the milk, which can be proactively prevented by ensuring a low bacterial plate count and SCC of raw milk, suppression of psychrotroph growth, an increase in the severity of heat treatment, and storage at temperatures higher or lower than 20–25°C. Also, the addition of polyphosphates inhibits the formation of β/κ gels (Datta & Deeth, 2001; Donato & Guyomarc'h, 2009). At higher temperatures (70–96°C), denaturation of α-lactalbumin occurs that forms complexes with aggregates of β-lactoglobulin. Denatured α-lactalbumin forms complexes with α_{s2}-casein and with MFGM at high temperatures (Corredig & Dalgleish, 1999; Jeanson *et al*., 1999; Considine *et al*., 2007). The residual native β-lactoglobulin content of heated milks is a reliable index for assessing the heat treatment of market milk up to ESL category, and more severe treatments cause excessive denaturation. Considering that the average β-lactoglobulin content of raw milk is 3300–3500 mg/L, the lowest value for pasteurised milk is 2600 mg/L, for high pasteurised milk 2000 mg/L and for ESL milk 1800 mg/L (Clayes *et al*., 2002; Mayer *et al*., 2010). Residual native α-lactalbumin content can be an index for more severe heat treatments. These are evident in Table 14.6, in which average values for market and experimental milks are presented. Special attention must be given

Table 14.6. Average residual β-lactoglobulin and α-lactalbumin contents (mg/L) in market and experimental heat-treated milks.

Milk category	β-Lactoglobulin	α-Lactalbumin
Raw	3000–4600	1060–1840
HTST pasteurised	1606–4140	850–1570
High-temperature pasteurised	790–3300	740–910
ESL	140–3680	850–1000
ESL microfiltered	3820	1060
UHT direct	150–1120	230–1130
UHT indirect	<170	82–482
Sterilised	<10	<10–50

Sources: based on data from Corzo *et al*. (1994b), Villamiel *et al*. (1997, 1999), Jeanson *et al*. (1999), Morales *et al*. (2000), Elliott *et al*. (2003, 2005), Feinberg *et al*. (2006), Lan *et al*. (2010), Mayer *et al*. (2010) and Lorenzen *et al*. (2011).

to the wide range of these average values, which is due to market sampling and mainly indicates that some products have been treated under more severe conditions than indicated on the packaging. Immunoglobulins and bovine serum albumin cannot serve as indices since they are very heat labile, especially the immunoglobulins; moreover, their initial concentrations in raw milk are low.

Cow milk allergy is a common food allergy in children up to 4 years old and results from IgE-mediated reactions against mainly caseins and β-lactoglobulin. Since heat treatment induces conformational changes, it is expected that allergenicity is affected. Although a decrease in the immune response to heat-treated milk proteins has been observed, it has to be considered that the heating conditions in some experiments were severe and unusual for drinking milk (El-Agamy, 2007; Morisawa *et al*., 2009; Taheri-Kafrani *et al*., 2009).

14.4.1.2 Enzymes

Numerous enzymatic activities can be detected in secreted milk, and these are called endogenous or native enzymes. They can affect the manufacture of dairy products and their stability during storage. Most of them are heat sensitive and cannot therefore deteriorate (low) pasteurised milk. However, there are notable exceptions, such as plasmin, which plays a key role in the instability of UHT milk during storage. In addition, some like alkaline phosphatase and lactoperoxidase are currently used as indicators of acceptable heat treatment, and evaluation of their activity in heat-treated milk is mandatory. Enzymes originate from many sources, such as blood, somatic cells and mammary gland, and are distributed to all milk phases, i.e. casein micelles, MFGM and milk serum (Moatsou, 2010, 2011). Their behaviour under heat treatment obeys first-order kinetics and is of particular importance for heat-treated milk (Table 14.7).

Both plasmin (EC 3.4.21.7) and plasminogen activators survive pasteurisation and are resistant to many UHT treatments. Thermal inactivation of the purified enzyme is achieved after heating at 80°C for 10 min at pH 7.0, whereas temperature–time combinations equivalent to treatment at 73°C for 40 min are necessary for its inactivation in milk. It is reversibly inactivated in the range 55–65°C and irreversible inactivation starts at temperatures above 65°C

Table 14.7. Behaviour of some important endogenous milk enzymes under heat treatments utilised for the production of milk for human consumption.

Enzyme	Source	Distribution in milk phases	Residual activity		
			(Low) pasteurisation	High-temperature pasteurisation	UHT
Plasmin (serine proteinase)*	Blood†	Casein micelles	High	High	+/–
Lipoprotein lipase	Mammary gland	Casein micelles	~0	0	0
Alkaline phosphatase	Mammary gland	Mainly MFGM	~0	0	0
Acid phosphatase		MFGM/SM	Considerable	Moderate	0
Lactoperoxidase	Mammary gland	Serum	60% of original	0	0
Xanthine oxidase (oxidoreductase)	Blood	MFGM	Enhanced	Traces	0
γ-Glutamyltransferase (transpeptidase)	Mammary gland	MMSM/MFGM	>50% of original	0	0
Catalase	Somatic cells	Cream/SM	~8% of original	0	0
Lysozyme	Lysosomes	Serum	Survives	+/–	0
Ribonuclease	Blood	Serum	Considerable		0
Superoxide dismutase		Serum	Survives	Survives	
Cathepsin D (aspartic proteinase)	Somatic cells	Acid whey	Survives partially	0	
Cathepsin B-like (cysteine proteinase)	Somatic cells		>20% of original		

*Part of a complex system of active and inactive forms.

†In the form of plasminogen.

SM, skimmed milk; MFGM, milk fat globule membrane; MMSM, membrane material in skimmed milk.

Source: based on data from Moatsou (2010, 2011).

according to first-order kinetics (Metwalli *et al*., 1998; Saint-Denis *et al*., 2001). Low pasteurisation enhances plasmin activity in milk by causing inactivation of plasminogen inhibitors, allowing the conversion of plasminogen to plasmin; only an appropriate UHT treatment (140°C for 15 s) can inactivate the plasmin system (Prado *et al*., 2006; Walstra *et al*., 2006). Nevertheless, the activation of bovine plasminogen observed at pasteurisation temperatures could be attributed to its denatured form, which is more readily activated by plasminogen activators than the native form (Burbrink & Hayes, 2006). The denatured β-lactoglobulin that occurs in milk during heat treatment destabilises plasmin due to thiol–disulphide interactions, whereas casein increases the heat stability of plasmin (Metwalli *et al*., 1998; Datta & Deeth, 2001; Chavan *et al*., 2011).

Lipoprotein lipase (EC 3.1.1.34) is heat sensitive and is almost inactivated by low pasteurisation (63°C for 30 min or 72°C for 15 s), its *D*-value at 70°C being 20 s. Therefore, it is not an agent that could potentially deteriorate market milk (Deeth, 2006; Walstra *et al*., 2006).

Alkaline phosphatase (ALP, EC 3.1.3.1) is an enzyme strongly connected to the heat treatment of milk because estimation of its activity is used to monitor the efficacy of (low) pasteurisation of milk. Inactivation of the enzyme ensures that all non-spore-forming pathogenic microorganisms present in milk, with reference to *Mycobacterium tuberculosis*, have been killed by the applied heat treatment. Under these conditions most but not all lactic acid bacteria and Gram-negative rods have also been killed. The *D*-value for ALP in bovine milk at 70°C is 33 s (Walstra *et al*., 2006) and at 60°C is 24.6 min (Clayes *et al*., 2002). A complication related to its use as a heat treatment indicator is its reactivation after UHT treatment of milk. No reactivation is observed in pasteurised milk and homogenisation before heat treatment reduces the extent of reactivation. Reactivation increases in the presence of Mg^{2+} and Zn^{2+}. The most important factor seems to be the SH groups of the whey proteins that are denatured under UHT conditions but not under low pasteurisation conditions. The SH groups of these whey proteins chelate heavy metals, which could otherwise bind to SH groups of the enzyme (Shakeel-Ur-Rehman *et al*., 2003).

Acid phosphatase (EC 3.1.3.2) increases substantially in mastitic milk and its residual activity after (low) pasteurisation is significant. Therefore, it could be a major problem in heat-treated milk. However, its optimum pH is about 4.0 and its low activity (in milk, only 2% that of ALP) does not favour its action. Its *D*-value at 100°C is 45 s, UHT treatment inactivates it, and in contrast to ALP it is not activated by Mg^{2+} (Shakeel-Ur-Rehman *et al*., 2003; Walstra *et al*., 2006).

Lactoperoxidase (LPO, EC 1.11.1.7) is a very important antimicrobial factor in raw milk and is one of the most heat-stable endogenous enzymes. Its *D*-value at 80°C is 4 s and at 71°C is 38.6 min (Clayes *et al*., 2002; Walstra *et al*., 2006). Complete inactivation is achieved at temperatures of 78°C or more for 15 s, and thermal inactivation in the range 69–73°C could be accurately described by a first-order kinetic model. Because of its heat stability under the pH conditions of milk, LPO activity is used as an indicator for heat treatments of milk more severe than low pasteurisation, i.e. high pasteurisation, although slow reactivation may be observed (Griffiths, 1986; Marín *et al*., 2003).

Xanthine oxidoreductase (XOR, EC 1.1.3.22) is very abundant in bovine milk; it comprises 20% of the protein content of the MFGM, which in bovine milk amounts to about 700 mg/L, so that bovine milk contains about 140 mg/L of XOR. The oxidation of XOR substrate yields very reactive superoxide ion and hydrogen peroxide. Therefore, it is considered a potent pro-oxidant and its presence at high levels in milk is related to spontaneous oxidative rancidity. Hydrogen peroxide produced by the action of XOR can serve as a substrate for LPO in its action as antibacterial agent (Harrison, 2006). Treatments that can damage or alter the MFGM (i.e. cooling, homogenisation or heat treatment) cause the release of XOR from the MFGM into the skimmed milk and this renders the enzyme more active. XOR activity is enhanced by cold storage of milk; after 24 hours, an increase in activity of 60–100% has been reported and crystallisation onset of milk fat can be related to the activation of XOR. Similarly, heating to 60–70°C activates it, whereas inactivation is accomplished at 73°C for 7 min and its *D*-value at 80°C is 17 s (Bhavadasam & Ganguli, 1980; Griffiths, 1986; Steffensen *et al*., 2004; Walstra *et al*., 2006).

γ-Glutamyl-transferase (EC 1.15.1.1) has been proposed as an indicator of milk heat treatment at temperatures above 77°C. After low pasteurisation treatment at 72°C for 15 s, more than 50% residual activity is observed; after treatment at 75°C for 15 s, less than 10% residual activity is observed. This residual activity is not affected by storage at 37 or 40°C. No activity is observed after heating at above 77°C for 15 s. Its kinetics during the heat treatments applied to milk are similar to that of LPO. For this reason γ-glutamyl-transferase has been proposed as an indicator of milk pasteurisation at temperatures above 77°C (McKellar & Emmons, 1991; Zehntner *et al*., 1995).

Catalase (EC 1.11.1.6) activity increases as SCC increase. About 26% of its activity is destroyed after milk thermisation at 60°C for 16 s. Low pasteurisation reduces its activity by 92%. Its *D*-value at 80°C is 2 s. The reactivation of catalase observed during refrigerated storage of heat-treated milk has been attributed to heat-resistant microorganisms (Griffiths, 1986; Hirvi & Griffiths, 1998; Walstra *et al*., 2006).

Lysozyme (EC 3.2.1.17) can survive in milk after heat treatment at 80°C for 15 s (Griffiths, 1986). The sensitivity of other enzymes has also been studied. Substantial RNase activity (EC 3.1.27.5) survives low pasteurisation but it is destroyed by UHT treatment (Griffiths, 1986). Superoxide dismutase (EC 1.15.1.1) is an antioxidant factor in milk acting against lipid oxidation and is extremely heat resistant. Its *D*-value at 80°C is 345 s, and inactivation is accomplished by heating at 75°C for 65 min (Hicks *et al*., 1979; Walstra *et al*., 2006). *N*-Acetyl-β-D-glucosaminidase (EC 3.2.1.30), a lysosomal enzyme released from somatic cells into the milk serum, is inactivated by (low) pasteurisation (Andrews *et al*., 1987). Cathepsin D (EC 3.4.23.5) is a lysosomal proteinase that is completely inactivated at 70°C for 10 min but survives partially pasteurisation (Hayes *et al*., 2001). Cathepsin B (EC 3.4.22.1) assumed activity observed in milk can potentially survive at more than 20% after heat treatment at 72°C for 30 s (Magboul *et al*., 2001).

Although enzymes from psychrotrophs do not belong to this category because they are not endogenous enzymes, they are intimately involved in milk heat treatments due to their extreme heat stability. The *D*-values of proteinase and lipase from *Pseudomonas* spp. are in the range 160–700 s at 130°C; at 150°C, ln k_0 and E_a of *Pseudomonas* lipase are ~22 and 83–91 kJ/mol, respectively (Walstra *et al*., 2006; de Jong, 2008). Some proteinases from psychrotrophs are susceptible to inactivation by heating at temperatures of about 55°C for some minutes, although according to *D*-values estimated from their heat inactivation at temperatures above 100°C the duration of heat treatment at moderate temperatures should be several hours (Griffiths *et al*., 1981). The growth of psychrotrophs must be controlled to avoid the presence of these enzymes in milk.

14.4.1.3 Vitamins

Raw milk is a source of many vitamins and particularly with respect to vitamins A, B_1, B_2, B_6 and B_{12}. Fat-soluble vitamin content is affected by the animal's nutrition and its environmental conditions (e.g. season of the year), while the concentration of water-soluble vitamins is more or less constant since they are mostly synthesised by the microbiological populations in the rumen. Apart from their nutritional significance, many of the vitamins (e.g. A, E and especially ascorbic acid) protect milk from undesirable oxidation reactions. The vitamin content in heat-treated milk, especially that treated at low pasteurisation conditions, is influenced by:

- the conditions of treatment, i.e. type of treatment, combination of temperature and time;
- the packaging type, i.e. exposure to light, oxygen permeability;
- the conditions of storage, i.e. temperature and duration.

Changes in riboflavin (vitamin B_2) and ascorbic acid (vitamin C) can, in turn, decrease the content of other vitamins and the appearance of off-flavours. Riboflavin is generally stable after heat treatment but is the most photosensitive vitamin. It absorbs in the visible range and acts as a photosensitiser by transferring the energy from light to highly reactive forms of oxygen, thus generating singlet oxygen in milk. Singlet oxygen induces reactions with unsaturated lipids, ascorbic acid (vitamin C), vitamin D, thiamine (vitamin B_1), pyridoxal (derivative of vitamin B_6) and folic acid. In addition, it reacts with sulphur-containing proteins and amino acids producing volatile compounds that are involved in the formation of off-flavours in milk exposed to light (Borle *et al*., 2001). A 2-hour exposure to sunlight of milk in clear glass bottles decreases the concentration of riboflavin by 20–80%, depending on the intensity of light. In general, artificial light from fluorescent tubes is poorer in energy compared with direct sunlight and therefore causes less damage (Bosset *et al*., 1995). Under these conditions, concomitant loss of ascorbic acid is observed, which is stable to light when riboflavin is not present (Biesalski & Back, 2002).

Two types of off-flavours are reported for milk exposed to visible or UV light: (i) a 'sunlight' or 'burnt feather' flavour that results from sulphur-containing protein fractions and (ii) a 'cardboard' flavour associated with the oxidation of lipid fractions appearing after prolonged exposure to light. These effects are induced by both natural and artificial light in the range 400–500 nm. The most important off-flavour is methional, caused by the photo-oxidation of methionine. Aluminium foil cartons (the best choice), or at least packaging with minimal transmission at 400–500 nm, protect milk from these phenomena as reported in section 14.3.5. Sterilised or UHT milk is less photosensitive than low pasteurised milk because the formation of free sulphydryl groups decreases the redox potential of milk (Kim & Morr, 1996; Rysstad *et al*., 1998; Borle *et al*., 2001; Rysstad & Kolstad, 2006; Walstra *et al*., 2006). Therefore, the photo-oxidation of riboflavin and the presence of oxygen determine the vitamin status and the organoleptic stability of heat-treated milks.

L-Ascorbic acid (ascorbate or vitamin C) plays an essential role as an antioxidant, acting as a biological reductant that can be regenerated when oxidised. It provides electrons for enzymes and oxidants; non-reactive intermediate free radicals are produced and its oxidation product, dehydroascorbic acid, is reduced by cells and can be reused. However, its hydrolysis in milk is promoted by photo-oxidation of riboflavin, leading to the irreversible formation of 1,3-diketogulonic acid and resulting in loss of the redox-buffering role of the previous reaction (Morrisey, 2002; Walstra *et al*., 2006). Reductions of vitamin C

depend on the source and intensity of the light, the exposure time, and the possible presence of metals and of oxygen even at trace levels (Bosset *et al*., 1995).

The effects of milk heat treatments on vitamins have been recently reviewed by Manzi and Pizzoferrato (2011). The meta-analysis of MacDonald *et al*. (2011) highlights significant variability among research studies due to various analytical methods, variable pasteurisation conditions utilised, and sample size. Depending on its severity, heat treatment significantly reduces the concentrations of vitamin C, folate, riboflavin, vitamin B_{12} and vitamin E. In particular, the duration of heat treatment was positively correlated with folate reduction, whereas vitamin C was inversely related to pasteurisation temperature. Although all heat treatments are described by the term 'pasteurisation', in fact the compiled data correspond to heat treatments of various intensities. The outcome of this survey is that (low) pasteurisation has practically no effect on both vitamin groups. Vitamin B_2 is almost unaffected by UHT treatment and this is also true for vitamin B_1. For vitamin B_6, losses of 4–35% are reported for UHT or close to UHT treatments, and under the same conditions vitamin B_{12} losses are about 30%.

The indices DRI and DAP are of particular importance for heat-treated milk. DRI is the degree of *cis*/*trans* isomerisation of retinol, which impairs the biological activity of this vitamin: the activity of the 13-*cis* isomer is about one-quarter that of all-*trans* retinol. Low levels of isomerisation (3–8%) are caused by pasteurisation, but UHT treatment has been reported to cause about 20% isomerisation. DAP is the degree of antioxidant protection in milk, constituted by α-tocopherol (vitamin E) and β-carotene, the levels of which are obviously lower in low-fat milks. DAP can be reduced by 10–20% by several types of pasteurisation (Sauvant *et al*., 2002; Manzi & Pizzoferrato, 2011). About 20% losses of vitamin A can be due to transparent packaging and the presence of oxygen, whereas losses of up to 96% have been observed in milk packaged in plastic pouches after 30 hours at 2200 lux (Bosset *et al*., 1995).

Treatments above (low) pasteurisation cause loss of vitamins at variable levels depending on the severity of heating, on the type of heating (direct or indirect), and on the storage period. Scott and Bishop (1986) presented a comparative study of the water-soluble vitamins in market pasteurised and UHT milks. Season of year did not influence complex B vitamins and vitamin C in pasteurised milk and the same was true for bottle or carton packaging. This latter finding was attributed to minimal exposure to light. UHT milks lost all ascorbic acid and contained only three-quarters of folate, about half of vitamin B_{12} and about one-third of vitamin C compared with pasteurised milk, whereas other vitamins were found at similar levels. Andersson and Öste (1994) reported significant losses of vitamin C during the storage of pasteurised milk but no significant changes in vitamins B_9 and B_{12}. Lorenzen *et al*. (2011) found no effect of direct or indirect heating or of a combination of microfiltration and pasteurisation as well as of storage period on the vitamin contents of ESL milks compared with HTST pasteurised milk. According to Walstra *et al*. (2006), the direct UHT method causes rather low degradation up to 20% of the heat-sensitive vitamins B_6, B_{12}, C and folate. However, after 3 months of storage 20–100% of vitamin C and folic acid and 20–50% of vitamins B_6 and B_{12} can be lost depending on light and oxygen exposure. Therefore heating intensity alone does not determine the vitamin status in market milks because the light and oxygen transmittance of packaging and the conditions of storage (i.e. transmission to light, temperature, duration) are equally important factors. The spectral emission characteristics and the intensity of light applied in market milk display cabinets and storage rooms must be taken into consideration in relation to milk packaging, so low-emission (blue-green, 350–550 nm) 'warm white' fluorescence tubes are appropriate (Bosset *et al*., 1995).

14.4.2 Formation of new substances

New substances found in heat-treated milk come from the reactions of lactose caused by heating. Two types of products belong to this category: those resulting from the isomerisation of lactose and those resulting from the reaction of lactose with proteins (i.e. Maillard reaction). Some of these products are appropriate indices for evaluating the heat load of processed milks, in a similar way to residual native proteins.

14.4.2.1 Isomerisation of lactose to lactulose

Lactulose is an isomerisation product of lactose that results from the conversion of glucose to fructose due to heating. It is considered as a prebiotic that promotes bifidobacterial proliferation in the intestine (Schuster-Wolff-Bühring *et al*., 2010; Seki & Saito, 2012). Lactulose provides a very good index for assessing the severity of milk heat treatment, since it is not detected in raw milk and in HTST pasteurised milk (Table 14.8). Moreover, it is rather stable during storage (Elliott *et al*., 2005; Cattaneo *et al*., 2008). Its formation follows pseudo zero-order kinetics and its concentration can be used to differentiate between milks treated by direct or indirect methods (Table 14.8; Clayes *et al*., 2002). Lactulose concentrations for UHT milk range from 100 to 600 mg/L, while milk with a lactulose content above 600 mg/L is characterised as sterilised. Mayer *et al*. (2010) report that a high limit for ESL milk could be 30 mg/L.

Epilactose comprises 10% of the total lactose isomers resulting from the isomerisation of glucose to mannose,

Table 14.8. Average lactulose (mg/L), furosine (mg/100 g protein) and hydroxymethylfurfural (HMF, µmol/L) content in market and experimental heat-treated milks.

		Products of Maillard reaction	
Milk category	**Lactulose[1]**	**Furosine[2]**	**HMF[3]**
Raw	0 or <50 or ~5	4–10	0–2 for total, 0–0.5 for free HMF*
HTST pasteurised	0 or 0–15	4–14	0–2.5 for total, 0.5 for free HMF*
High-temperature pasteurised	0–80	10–35	
ESL		11–260 or 10–22	
ESL microfiltered		8	
UHT	50–850, mostly up to 350 for direct and 190–830 for indirect heating	16–485, mostly up to 180 for direct and 40–430 for indirect heating, increasing with storage time	Direct: 3–6.5 for total, 0.2–1.1 for free HMF Indirect: 0–16 for total, 0–1.7 for free HMF or 0–2.5 for total, 0.5 for free HMF*
Sterilised	1080–1400	250–440	12–22

*Total HMF corresponds to the Amadori product (see text) and is an index of the initial Maillard reaction, whereas free HMF is an index of advanced Maillard reaction stages.

Sources: based on data from

[1]Andrews *et al*. (1987), Olano *et al*. (1989), Mayer *et al*. (1996), Birlouez-Aragon *et al*. (1998), Villamiel *et al*. (1999), Morales *et al*. (2000), Elliott *et al*. (2003, 2005), Marconi *et al*. (2004), Feinberg *et al*. (2006), Cattaneo *et al*. (2008), Lan *et al*. (2010).

[2]Corzo *et al*. (1994a, b), Clawin-Rädecker & Schlimme (1995), Pellegrino *et al*. (1995), Tirelli & Pellegrino (1995), Van Renterghem & De Bloc (1996), Birlouez-Aragon *et al*. (1998), Jeanson *et al*. (1999), Villamiel *et al*. (1999), Elliott *et al*. (2003, 2005), Feinberg *et al*. (2006), Fenaille *et al*. (2006), Cattaneo *et al*. (2008), Lan *et al*. (2010), Mayer *et al*. (2010) and Lorenzen *et al*. (2011).

[3]Morales *et al*. (1995, 1996, 2000), Morales & Jiménez-Pérez (1999), Elliott *et al*. (2003).

and can therefore be detected only in sterilised milks. Furthermore, the degradation of disaccharides results in an increase in free galactose, which isomerises to tagatose under sterilisation conditions (López-Fandiño & Olano, 1999).

14.4.2.2 Maillard reaction products

In general, the activation energy (E_a) for Maillard reactions is 100–180 kJ/mol and the Q_{10} at 100°C ranges from 2 to 4.5 (Walstra *et al*., 2006; de Jong, 2008). Furosine is related to the first stages of Maillard reactions and is the steady product produced by the acid hydrolysis of unstable lactulosyl-lysine. During milk storage, an increase of 7 mg/100 g protein is reported every 10 days (Pellegrino *et al*., 1995; van Boekel, 1998; Elliott *et al*., 2005) (Table 14.8). In general, furosine is a more convenient index compared with lactulose, since it covers nearly all types of heat treatments. An upper limit of 8 mg/100 g protein is proposed for (low) pasteurised milk and 20 and 250 mg/100 g protein for high-temperature pasteurised and UHT milks, respectively, whereas a limit of 20 mg/100 g milk is proposed for ESL milk (Clayes *et al*., 2002; Mayer *et al*., 2010).

Hydroxymethylfurfural (HMF) has also been used for the assessment of milk heat treatments ranging from UHT to sterilisation, although very low amounts are reported for raw milk, which is due to sample digestion prior to analysis (Morales *et al*., 1995, 1996; Morales & Jiménez-Pérez, 1999; Table 14.8). It is the result of two types of reactions that occur during heating: (i) isomerisation and sugar degradation reactions and (ii) decomposition of lactulosyllysine during Maillard reactions. It is expressed as free HMF, which corresponds to the first pathway, and as total HMF from the second pathway, i.e. Amadori products yielded by a sequence of rearrangement reactions of the Schiff base formed between the aldehyde group of glucose and the amino group of lysine, and basically corresponds to Maillard reaction conditions (Clayes *et al*., 2002; Walstra *et al*., 2006). Also, carboxymethyllysine produced from Amadori products in the presence of oxygen has been proposed as an index for very severe heat treatments (van Boekel, 1998; Fenaille *et al*., 2006).

14.4.3 Others

During milk heat treatment, dehydroalanine can be formed from serine and cysteine and which reacts with lysine to form lysinoalanine. Cattaneo *et al*. (2008) suggest that the evaluation of lysinoalanine can be an index for the storage conditions of UHT milk.

14.5 CONCLUSIONS

The various treatment strategies applied in the production of milk for human consumption, combining heat treatment and packaging and occasionally filtration techniques along with acceptable production and handling conditions of raw milk, ensure consumer safety and the quality of products during storage. However, the quality of market milk also depends on the storage conditions, and therefore factors like the cold chain and exposure of the product to light or oxygen must be considered part of the treatment. (Low) pasteurised milk is safe and retains almost all the nutritional and organoleptic qualities of raw milk. The production of market milk with characteristics close to those of pasteurised milk but with rather longer shelf-life (ESL milk) is a current objective of the dairy industry and dairy research. In this respect, efficient direct heating systems, especially of the infusion type, are very successful because the instantaneous heating and cooling that they provide cause less damage but have the same microbiological effect compared with indirect methods. Packaging affects both shelf-life and product stability. Shelf-life can be extended using ultraclean or aseptic packaging under aseptic filling, which is part of UHT treatments and is also utilised in some ESL processes. Milk stability is impaired by exposure to light and oxygen, which damage the complex system of milk vitamins and induce the formation of off-flavours from milk lipids and protein fractions, a process that starts with the photo-oxidation of riboflavin. The storage conditions of heat-treated milk, especially of the UHT category, are very important because deterioration of product and destruction of vitamins can occur. In fact, storage conditions may have a more severe impact on some vitamins than the heat treatment itself. Finally, assessment of the heat treatment of market milk can be achieved using a combination of indices that reflect the heat-induced changes in milk. The relation of these indices with some particular components like vitamins is a useful tool for the classification of heat-treated milks according to their nutritional characteristics.

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15
Sensory and Flavor Characteristics of Milk

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15.1 INTRODUCTION

Milk from ruminant species is one of the main components of the human diet, especially in Western countries, because of its balanced nutritional composition and its versatility to be transformed into a great diversity of products. Although cows' milk is the main contribution to global milk production, in some countries, such as India and Pakistan, the majority of milk is produced by buffaloes, whereas in the Mediterranean region the production of milk by small ruminants such as sheep and goats is of considerable importance compared with that of cows' milk (Manfredini & Massari, 1989; Fox, 2011). Distinct differences in physicochemical properties have been reported among milks from ruminant species, and between milks from ruminant and monogastric species (Doreau & Martin-Rosset, 2002; Park & Haenlein, 2006). These compositional differences lead inevitably to distinctive sensory characteristics. Milk as discussed here refers to cows' milk unless otherwise stated.

Among sensory characteristics of milk, flavor is one of the most important attributes for acceptability and preference by consumers (Thomas, 1981; Kim & Morr, 1996). It is also a key attribute for obtaining quality products since it is the common and basic ingredient for many formulated dairy and non-dairy foods (Drake *et al*., 2003; Lloyd *et al*., 2009a). Typical milk flavor is the result of a delicate balance of a wide number of compounds, some of them present at very low concentrations (Nursten, 1997). Milk flavor depends on the metabolism of the animal and interactions between the animal and its environment (Toso *et al*., 2002), and thus variations in this attribute are expected (Thomas, 1981). These changes can lead to different defects known as off-flavors, which are associated with an unbalanced volatile profile.

It is well known that the smell of raw milk is typical for each ruminant species, which is considered dependent on some quantitative differences in the volatile profile and on the presence of specific compounds associated with each type of milk (Moio *et al*., 1993a; Toso *et al*., 2002). Cow milk flavor has been extensively studied but research on flavor of small ruminant milk is scarce (Carunchia Whetstine & Drake, 2006).

From very early times, the dairy industry and research in dairy science and technology have made enormous efforts to obtain safe dairy products and to extend their shelf-life and improve quality, including quality consistency. Nowadays, these efforts have doubled as new products have to be developed to satisfy the growing demand of consumers, who require both safe and healthy products and appealing sensory properties (Chapman *et al*., 2001).

Thermal treatments are extensively applied to raw milk to reach different objectives: (i) to ensure food safety by destroying pathogenic microorganisms, and (ii) to stabilize the product by inactivating enzymes and killing spoilage microorganisms, which prolongs shelf-life and allows the products to be stocked at room temperature (de Wit & Nieuwenhuijse, 2008; Hougaard *et al*., 2011). Pasteurization,

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ultrapasteurization (UP), ultra-high-temperature (UHT) process, in-container sterilization, and spray drying are the most extended thermal treatments in the dairy industry. The quality of thermally treated milks is greatly determined by the onset of unpleasant flavors which occur when processing variables deviate from appropriate conditions or when more stringent conditions of temperature/time are applied. The incidence of objectionable cooked flavor in pasteurized milk decreased considerably with the adoption of continuous processing methods such as high-temperature short-time pasteurization (HTST) (Thomas, 1981). UHT sterilization can promote strong sulfurous, cooked, cabbage-like flavors in milk, thus limiting its acceptance (Vazquez-Landaverde *et al*., 2006a). A precise control of processing conditions can reduce the appearance of this defect. Sensory changes in processed milk can also occur as a consequence of other factors including raw milk quality, post-processing contamination, storage conditions, and the use of certain packaging material that accelerate enzymatic and chemical reactions (Celestino *et al*., 1997a, b; Simon & Hansen, 2001a; Solano-Lopez *et al*., 2005; Smet *et al*., 2008). Specific volatile compounds have been linked to typical defects that develop in heated milks through storage, and the physical agents or chemical and enzymatic reactions that cause them have been established. For example, flavors of UHT milk described as cooked and cabbagey appear during the first stage of storage, and their intensity is dependent on the presence of volatile sulfides liberated via heat denaturation of the protein β-lactoglobulin (Simon & Hansen, 2001a; de Wit & Nieuwenhuijse, 2008). Short-chain free fatty acids released by lipases from milk fat can cause rancid flavors (González-Córdova & Vallejo-Cordoba, 2001). Stale flavors can be produced by several compounds originating mainly from non-enzymatic browning in milk powder (Karagül-Yüceer *et al*., 2002) or from lipid oxidation such as methyl ketones, linear-chain aldehydes, and sulfur compounds in UHT milk (Jeon *et al*., 1978; Rerkrai *et al*., 1987).

In recent years, non-thermal technologies have been explored to meet new demands of consumers about fresher and more natural products. They are required to ensure food safety with a minimum change in sensory characteristics. Technologies such as microfiltration, ultrasonication, pulsed electric field, microwave, and high pressure, alone or in combination with minimal thermal treatments, have been applied to milk. The effect of these promising technologies on volatile profile and flavor is still being investigated, although the preliminary results are encouraging (Trujillo *et al*., 2002; Clare *et al*., 2005; Vazquez-Landaverde *et al*., 2006b; Riener *et al*., 2009; Zhang *et al*., 2011).

Over recent decades large amounts of information have been accumulated on flavor and volatile compounds of milk from different species. Factors that can cause variations in the volatile profile and sensory characteristics of milk, from its biosynthesis to its sale, have been investigated. Most attention has been focused on off-flavor due to the economic impact that it implies. Other topics, such as the mechanisms and metabolic pathways that explain the origin of volatile compounds, or the impact of new milk-processing technologies are more recent research approaches or have not been elucidated.

15.2 SIGNIFICANCE OF FLAVOR AND OFF-FLAVOR ON MILK QUALITY: SENSORY AND INSTRUMENTAL METHODS

Milk flavor is one of the most important attributes for acceptability and preference by consumers (Thomas, 1981), being also a key attribute to obtain quality products (Drake *et al*., 2003; Lloyd *et al*., 2009a). Off-flavors have always been a major control problem for the dairy industry (Thomas, 1981), decreasing the sensory quality and economic value of products (Karagül-Yüceer *et al*., 2002). In addition, they can be a contributing factor in the decline of per capita consumption of milk (Leong *et al*., 1992).

Flavor is a complex sensation in which aroma plays the most important role, being mainly perceived by the interactions of the volatile components with the receptors of the olfactory epithelium in the nasal cavity (Nursten, 1997). Flavor cannot be measured directly by instrumental methods because it is the result of an interaction between food and consumer. Thus, milk quality evaluation has been commonly carried out by human assessment. Much research has attempted to develop objective tests for quality analysis (Horimoto & Nakai, 1998), since sensory methods are subject to errors from differences among individual assessments. Only the selection of an appropriate test and its correct application as well as accurate data interpretation leads to reproducible and relevant results (Drake, 2007). Excellent reviews on different aspects of sensory analysis of dairy foods, such as description of methods, when and how to use them and the information that can be obtained in each case, are available (Drake, 2004, 2007). Among sensory methods, descriptive sensory analysis is extensively employed in dairy products evaluation to identify and measure those attributes that best characterize their sensory properties. Its application requires both defined terms for sensory attributes and descriptors and trained panelists or judges, who can assess a certain product quality and detect changes due to off-flavors (Biolatto *et al*., 2007). Possible terms with references and accepted definitions for attributes and descriptors can be achieved through the development of a language (Drake & Civille, 2003). Descriptive sensory evaluation and flavor lexicons for fluid milk (Watson & McEwan, 1995; Chapman *et al*., 2001), milk powders

(Drake *et al*., 2003), dairy ingredients (Drake *et al*., 2003), and cheese (Muir *et al*., 1995; Drake *et al*., 2005; Liggett *et al*., 2008) have been proposed. A widespread lexicon addressed to off-flavor of milk was reported by the Committee on Off-Flavor Nomenclature and Reference Standards of the American Dairy Science Association (Shipe *et al*., 1978). This committee proposed seven categories of off-flavors (Table 15.1). In the USA and other Western countries these terms have been used extensively in formulating 100-point score cards for the evaluation of milk or cheeses and other dairy products in annual state and national judging competitions and products contests for high school [4-H youth clubs, FFA (Future Farmer of America) associations], collegiate and adult contestants, and product exhibitors (Nelson & Trout, 1964; Haenlein, 2000). The awarding of prizes and championship trophies for best-quality entries is inspiring for product quality improvement and very popular.

Objective tests for milk quality evaluation are not necessarily associated with consumer acceptance or preference. For this reason, consumer tests are widely used to determine both acceptability and shelf-life of a product (Drake, 2007).

Milk flavor is due to a complex mixture of volatile components in a specific matrix, consequently the analysis of the volatile fraction by instrumental methods becomes another important criterion in quality evaluation of dairy products (Nursten, 1997; Povolo *et al*., 2007), since it allows to identify objectively the presence and intensities of compounds linked to food flavor (Horimoto & Nakai,1998). Headspace techniques (static and dynamic headspace), solid-phase microextraction, distillation and/or solvent extraction (solvent-assisted flavor evaporation, simultaneous distillation extraction, etc.) are widely applied to characterize the aromatic fraction of milk and to evaluate the impact of different factors on the volatile profile.

The study of volatile fractions of milk by instrumental analytical methods is complex for several reasons: the majority of analytes are present at very low concentrations (Imhof & Bosset, 1994; Bendall, 2001) and the heterogeneous nature of the matrix of milk makes isolation difficult (Friedrich & Acree, 1998; Havemose *et al*., 2007). As many components are highly sensitive to heat, temperature extraction is critical to avoid generation of artifacts and to preserve the original flavor. In addition, it is not an easy task to establish the role of a single compound on overall flavor or to relate volatile compounds with desirable or undesirable sensory attributes. Finally, the concentration of a compound is not necessarily a measure of its sensory impact (Drake & Civille, 2003).

The onset of techniques combining olfactometry (O) and gas chromatography (GC) has enabled, at least partially, to overcome these difficulties (Friedrich & Acree, 1998). Their application to fresh and thermally treated milk samples has allowed identifying those components with a strong impact on flavor and to relate them with the characteristic flavor of each type of milk (Table 15.2). Electronic noses have been more adequate to distinguish normal samples from those with off-flavor (Marsili, 1999) or to monitor rancidity during storage (Capone *et al*., 2001).

Statistical tools are essential to find a correlation between sensory attributes and individual volatiles; they range from simple correlations to multivariate analyses such as factor analysis (FA), cluster analysis (CA), principal components analysis (PCA), and principal component similarity analysis (PCS) (Horimoto & Nakai, 1998).

15.3 MILK FROM RUMINANT SPECIES

15.3.1 Volatile profile and sensory characteristics of fresh milk

Sensory characteristics of milk from ruminants (cows, ewes, goats, etc.) differ from one species to another. Fresh cows' milk of overall good quality has a bland and clean but distinctive flavor, which has been described as a slightly salty-sweet taste and delicate aroma (Thomas, 1981; Bendall, 2001). The salty-sweet taste has been mainly attributed to milk salts and lactose but in fact, the aroma makes the most important contribution to flavor (Marsili, 2011). In addition, the sensory perception of bovine milk is significantly impacted by the pleasant mouth feel and aftertaste, which is due to the emulsion of milk fat (Badings & Neeter, 1980; Francis *et al*., 2005). However, since bovine milk possesses this bland and soft flavor, any deviation in this typical characteristic is readily perceived by consumers (Thomas, 1981; Shiratsuchi *et al*., 1994a). In contrast, the milk of small ruminants such as sheep and goats tends to have a more intense aroma characterized by waxy and animal notes. These attributes are not present in bovine milk. Milk fat composition plays a key role by defining its flavor (Carunchia Whetstine & Drake, 2006).

Both raw and pasteurized milk are usually considered "fresh milk" since the conventional pasteurization process (HTST) does not significantly alter the flavor or volatile compound profile (Moio *et al*., 1994; Nursten, 1997; Bendall, 2001; de Wit & Nieuwenhuijse, 2008). Consequently, in this chapter we will refer to raw and HTST pasteurized milk as "fresh milk." For few pasteurization processes, however, differences have been reported between raw and pasteurized milk: analysis of sheep milk by a sensory panel revealed that HTST pasteurized milk and untreated milk were not significantly different, but batch-pasteurized milk was described as muttony (Young, 1986). Another example: instant infusion pasteurization is often considered as a gentle process, but

Table 15.1. Categories of off-flavors proposed by Shipe *et al.* (1978).

Categories	Definition	Descriptive terms	References
Heated	Flavors that result from changes in milk components produced by thermal treatments	Cooked, heated, caramelized, scorched	Shipe *et al*. (1978)
Oxidized	Flavors produced from a reaction between molecular oxygen and polyunsaturated fatty acids, which can be induced by certain metals or light. In addition, this process may also occur spontaneously. Milk from some cows develops this defect so quickly, which is known as "spontaneous oxidation"	Oxidized, cardboardy, metallic, tallowy, oily, fishy	Shipe *et al*. (1978) Barrefors *et al*. (1995) Timmons *et al*. (2001)
Light-induced	Defects produced by light action on certain milk components. It has two distinct causes: one a burnt or sunlight flavor attributed to photodegradation of proteins and amino acids, which develops rapidly during the first days of storage and therefore become noticeable early, and a second component defined as metallic or oily that does not dissipate and becomes dominating in milk, similar to oxidized flavor, and attributed to lipid oxidation. In this case, photo-oxidation occurs in milk in the presence of a photosensitizer such as riboflavin, which excites oxygen to its singlet state by producing free radicals that can react with unsaturated fatty acids	Light, sunlight, activated, burnt, scorched, cabbage, old vegetable oil, cardboard, goat, metallic	Shipe *et al*. (1978) Jenq *et al*. (1988) van Aardt *et al*. (2001) Chapman *et al*. (2002) Moyssiadi *et al*. (2004) Havemose *et al*. (2004)
Lipolyzed	Flavors produced by hydrolytic release of free fatty acids from triglycerides by lipolytic enzymes. The particular action of native milk lipase (lipoprotein lipase) on cold stored milk fat is a process known as "spontaneous lipolysis"	Rancid, butyric, goaty, soapy	Shipe *et al*. (1978) Cartier & Chilliard (1990)
Microbial	Abnormal flavors that result from spoilage microorganisms or enzymes, which cause protein and fat degradation of milk	Acid, malty, fruity, putrid, unclean, sour, bitter	Shipe *et al*. (1978)
Transmitted	Flavors that may arise by passage of substances from the cow's feed to the mammary glands. This transfer may be via the respiratory and/or digestive system and bloodstream. In this category are also considered those flavors related to the migration into the milk of degradation products of plastic or residual solvent employed in packaging materials	Feed, weed, cowy, barny Unpleasant plastic	Shipe *et al*. (1978) Marsili (2011) Leong *et al*. (1992)
Miscellaneous	Flavors that either cannot be attributed to a specific cause or specifically defined in sensory terms	Absorbed, astringent, bitter, chalky, chemical, foreign, lacks freshness, salty	Shipe *et al*. (1978)

Source: based on data from Shipe *et al*. (1978).

Table 15.2. Main odor-active compounds reported in different types of milks.

Compounds	Odor descriptors*	Type of milk	References
Ethyl butanoate	Fruity, sweet, banana, fragrant	Bovine raw milk Ovine raw milk Caprine raw milk Buffalo raw milk	Moio *et al*. (1993a, 1994, 1996)
Ethyl hexanoate	Fruity, pineapple, apple, unripe fruit	Bovine raw milk Ovine raw milk Caprine raw milk	Moio *et al*. (1993a, 1994, 1996)
Heptanal	Green, sweet, herbaceous	Ovine raw milk Buffalo raw milk	Moio *et al*. (1993a, 1996)
Indole	Fecal, putrid, musty, floral in high dilution	Buffalo raw milk Caprine raw milk Ovine raw milk	Moio *et al*. (1993a, 1996)
Nonanal	Sweet, floral, green, grass-like	Buffalo raw milk Ovine raw milk Pasteurized bovine milk	Moio *et al*. (1993a, 1994, 1996)
1-Octen-3-ol	Mushroom-like	Buffalo raw milk Ovine raw milk Pasteurized bovine milk	Moio *et al*. (1993a, 1996)
Dimethylsulfone	Sulfurous, hot milk, burnt	Bovine raw milk Ovine raw milk Buffalo raw milk Pasteurized bovine milk UHT bovine milk	Moio *et al*. (1993a, 1994)
Hexanal	Freshly cut grass, green	Pasteurized bovine milk	Moio *et al*. (1994)
2-Heptanone	Blue cheese, spicy	UHT bovine milk	Moio *et al*. (1994)
2-Nonanone	Mustard like, spicy	UHT bovine milk	Moio *et al*. (1994)
2-Undecanone	Vegetable, floral, rose-like	UHT bovine milk	Moio *et al*. (1994)
Benzothiazole	Burning smell, rubbery	UHT bovine milk	Moio *et al*. (1994)
2-Tridecanone + δ-decalactone	Peach-like, floral	UHT bovine milk	Moio *et al*. (1994)

*Based on data from Moio *et al*. (1993a, 1994) and Friedrich & Acree (1998).

sensory properties of bovine milk subjected to this treatment were described by negative attributes such as cardboard, sour, and plastic, and its volatile fraction composition differed from HTST pasteurized milk (Hougaard *et al*., 2011).

Compounds involved in fresh milk flavor do not derive from a single source in the food. They can come from direct transfer from the cow's fodder, by absorption from the digestive tract (i.e. rumen and/or intestine) into the blood and thence to peripheral tissues such as the mammary gland. A second possible absorption is via the pulmonary route. In this case, compounds present in the air are inhaled by the ruminant or gases from the rumen absorbed in the lungs into the blood and from there they diffuse to the mammary gland. Finally, flavor compounds can be formed by metabolism (endogenous synthesis) in the rumen and/or by metabolic processes in the liver or mammary gland from carbohydrates, amino acids, fatty acids, and other compounds present in the fodder (Honkanen *et al*., 1964; Urbach, 1990a; Moio *et al*., 1996). In addition to those components naturally present in raw milk, a wide number of compounds can be produced by chemical or enzymatic reactions before and during dairy processing or induced during storage (Calvo & de la Hoz, 1992).

The most extensive research work available on volatile compounds of fresh milk was performed by Moio *et al*. (1993a, b, 1994, 1996), who analyzed milks from different ruminant species: cow, sheep, goat, and buffalo. In these studies, more than 80 volatile compounds belonging to different chemical classes, such as alcohols, ketones, esters, acids, sulfur, and nitrogen compounds, and aliphatic and aromatic hydrocarbons were detected. Volatile compounds identified were similar for the four

ruminant species, so results suggested that typical flavor for each ruminant milk mostly relied on quantitative differences. Regardless of this, certain compounds are considered as characteristic of each ruminant milk.

Among carbonyl compounds, methyl ketones, diketones, straight-chain aldehydes, branched-chain aldehydes, and aromatic aldehydes are frequently found in milk (Imhof & Bosset, 1994; Bendall, 2001; Toso *et al*., 2002; Francis *et al*., 2005). Quantitatively, aldehydes are a main group of compounds in the volatile profile of milk and some of them seem to be characteristic of milk from specific animal species (Moio *et al*., 1993b, 1996; Toso *et al*., 2002). For example, 3-methylbutanal was found only in buffalo milk whereas benzaldehyde and phenylacetaldehyde were not present in caprine milk (Moio *et al*., 1993b). Similarly, the importance of aldehydes in fresh milk flavor differs from one species to another. Nonanal is considered a key compound only in the aroma of raw buffalo milk (Moio *et al*., 1993a) and heptanal makes a special contribution to the aroma of ewes' milk (Moio *et al*., 1996). Differences in the levels of aldehydes have been observed from milks produced by sheep and cows subjected to different diets (Moio *et al*., 1996; Toso *et al*., 2002). In fact, aldehydes provided one of the best discriminant criteria for grouping milks according to type of forage in the ration (Toso *et al*., 2002), which can be very important in identification and validation of origin of dairy products under European protection law.

At low concentrations (approximately 10–40 ppb), aldehydes are associated with pleasant herbaceous notes, but at higher concentrations they can give a penetrating and unpleasant off-flavor (Moio *et al*., 1996). In particular, the contribution of hept-*cis*-4-enal to milk flavor has been investigated. On the basis of its low odor threshold value, its level found in milk (approximately 50 pg/g) and its odor quality has been recognized as an important odorant (Bendall & Olney, 2001).

Unlike the aldehydes, ketones have been largely reported as minor compounds in the raw milk of different ruminants (Moio *et al*., 1993b, 1996). However, other studies have pointed to the ketones as a quantitatively important group in bovine milk (Contarini *et al*., 1997; Toso *et al*., 2002). Different results can be explained by the application of different extraction techniques; however, evidence that ketones are important contributors to fresh milk flavor has not been provided so far. Among ketones, diacetyl has attracted interest because of its typical buttery notes. The role of this compound on overall aroma has not been elucidated as information about its level in unfermented milks is scarce and contradictory. For example, Scanlan *et al*. (1968) reported a concentration of diacetyl in raw milk below threshold value whereas Macciola *et al*. (2008) detected the presence of significant amounts.

Ketones and aldehydes in milk have more than one origin. Methyl ketones with low molecular weight such as propanone and butanone mainly result from the cow's metabolism. Milk fat contains fatty acids which are precursors of methyl ketones with odd-number carbon atoms such as 2-pentanone and 2-heptanone. A mechanism of β-oxidation and decarboxylation of saturated fatty acids or decarboxylation of β-ketoacids naturally present in milk has been proposed (Contarini *et al*., 1997). Diacetyl could be transferred to the milk from forage eaten by the cow (Scanlan *et al*., 1968), directly produced during lactation or it could be formed by enzymatic reaction of certain milk compounds (Macciola *et al*., 2008). Saturated and unsaturated straight-chain aldehydes can be formed by autoxidation of unsaturated fatty acids (Contarini *et al*., 1997). Branched-chain aldehydes and aromatic aldehydes can derive from amino acid catabolism by enzymatic activity (Moio *et al*., 1993b).

Alcohols have been found in small quantities in fresh milk. Their prevalence varies among species, from 1.5% of neutral volatile fraction in bovine milk to 5% in buffalo milk (Moio *et al*., 1993b). Primary and branched-chain alcohols are the main alcohols in milk; they are probably derived from the respective aldehydes by enzymatic reduction (Imhof & Bosset, 1994; Toso *et al*., 2002). During storage of raw milk at refrigeration temperatures, an increased level of alcohols attributed to reduction of carbonyl compounds has been observed (Urbach, 1990b).

Alcohols occur in low quantities in fresh milk and their relative contribution to flavor is considered secondary (Toso *et al*., 2002). Only the 1-octen-3-ol, derived from unsaturated fatty acid degradation, was identified as key odorant in buffalo and ovine milk (Moio *et al*., 1993a).

Esters have been reported as the main group of the neutral volatile fraction of milks from different species (Moio *et al*., 1993b). Ethyl butanoate and ethyl hexanoate are among the most powerful flavor compounds in freshly secreted bovine, caprine, and ovine milk (Moio *et al*., 1993a), which confer a fruity note to the milk (Moio *et al*., 1996). In contrast, a secondary role was observed for esters in pasteurized milk, probably due to their destruction during thermal processes (Moio *et al*., 1994). The origin of esters in raw milk is not fully elucidated. A biosynthesis within the mammary gland or after milking by bacterial activity is believed possible (Moio *et al*., 1993b; Toso *et al*., 2002).

Sulfur and nitrogen compounds are also characteristic compounds of raw and processed milk. Among them, hydrogen sulfide, methanethiol, dimethyl sulfide, carbon disulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl sulfone, 2-acetyl-1-pyrroline, benzothiazole, 2-isobutyl-3-metoxypyrazine, indole, and skatole can

be mentioned (Moio *et al*., 1993b, 1996; Imhof & Bosset, 1994; Al-Attabi *et al*., 2008).

Dimethyl sulfone, a typical sulfur compound, has been identified as a major odorant from a quantitative viewpoint in milk from different mammals, with 25% of the volatile fraction in bovine, caprine, and ovine milk, and only 4% in buffalo milk (Moio *et al*., 1993b). Dimethyl sulfone aroma is described as hot milk, leather, and bovine sweat-like (Vazquez-Landaverde *et al*., 2006a), and its role on milk flavor is controversial: data on threshold values are lacking and wide variations of concentrations have been reported for different diets or thermal treatments (Moio *et al*., 1993a, b, 1994, 1996). On the other hand, dimethyl sulfide, dimethyl trisulfide and methanethiol, which occur at low concentrations, are known to be powerful flavor compounds in milk (Vazquez-Landaverde *et al*., 2005, 2006a).

The presence of sulfur-containing compounds in milk is a consequence of feed given to ruminants but they can also be formed from protein-bound cysteine and methionine, being β-lactoglobulin the main protein involved (Vazquez-Landaverde *et al*., 2006a; de Wit & Nieuwenhuijse, 2008). Catabolism of methionine produces dimethyl sulfide and methanethiol. Methanethiol can be further oxidized to dimethyl disulfide and dimethyl trisulfide (Bendall, 2001; Vazquez-Landaverde *et al*., 2006a). Dimethyl sulfone is probably produced from dimethyl sulfide oxidation or as a result of the cow's metabolism (Moio *et al*., 1993b).

Nitrogen compounds appear to be essential for the aroma of different raw milks. The quantities reported are higher in ovine, caprine, and buffalo milk than in bovine milk, and they can vary widely according to the feed (Moio *et al*., 1993b, 1996). In particular, indole is recognized for its odorant properties: it has a low threshold value and can contribute to milk aroma (Moio *et al*., 1993a, b). At very low levels indole is characterized by a delicate smell reminiscent of jasmine, whereas at higher concentrations it is associated with unpleasant notes of feces (Moio *et al*., 1996).

Several free fatty acids, mainly linear-chain fatty acids of even carbon atoms from C4:0 to C10:0 have been identified in bovine fresh milk, but they are of minor relevance to flavor (Scanlan *et al*., 1965). In contrast, short- and medium-chain fatty acids (C6:0 to C12:0) are the main contributors to sheep and goats' milk flavor and specifically, branched-chain fatty acids are responsible for the characteristic waxy and animal notes (Carunchia Whetstine & Drake, 2006).

Although certain fatty acids can result from amino acid catabolism or lactose degradation, most are derived from triglyceride hydrolysis by lipolytic enzymes. Thus, the particular milk fat composition and lipolytic activities that characterize milk of each ruminant species determines the lipolysis extent and the type of released fatty acid, which in turn impacts greatly on flavor. Little or no lipolysis should occur in fresh milk (Escobar & Bradley, 1990). Therefore, the levels of free fatty acids should be below the detection threshold of rancid off-flavor.

Terpenes, hydrocarbons, and phenolic compounds are normal constituents of fresh milk. Certain terpenes and hydrocarbons are related to diet: it is well-known that monoterpenes and sesquiterpenes can be readily transferred from forages into milk fat, with only minor changes (Viallon *et al*., 2000). Terpenes are products of secondary metabolism of plants, recognized for their disinfectant (medicinal) and odorant (spice) properties. Natural highland pastures rich in dicotyledons generally contain higher quantity and wider diversity of terpenes than the lowland pastures rich in *Gramineae* (Mariaca *et al*., 1997; Viallon *et al*., 2000); they are also more abundant in fresh grass than in hay (Zeppa *et al*., 2004). This profile of terpenes is reflected in milks from cows fed with these pastures and, consequently, terpenic compounds can help to characterize dairy products obtained from milk of ruminants fed different forages, in different seasons, or from different geographical areas (Dumont & Adda, 1978; Viallon *et al*., 2000; Bugaud *et al*., 2001; Zeppa *et al*., 2004). Sesquiterpenes, especially, are considered promising biochemical markers that could be used to link a dairy product to its geographical region (Fernandez *et al*., 2003; Tornambé *et al*., 2006; Povolo *et al*., 2007; Abilleira *et al*., 2011). Unlike the hydrocarbons which probably do not contribute to aroma due to the low concentrations in milk and the high perception thresholds (Moio *et al*., 1993b), terpenes can influence the milk flavor since they are often characterized by a fruity, herbaceous or resinous odor (Addis *et al*., 2006).

A wide variety of phenolic compounds, such as phenol, *o*-, *m*-, and *p*-cresol, 2- and 4-ethyl phenol, thymol, and carvacrol, have been identified in fresh milk (Lopez & Lindsay, 1993; Moio *et al*., 1993b, 1996; Bendall, 2001). A high proportion of them is in the form of metabolic conjugates (glucuronides, sulfates and phosphates) (Lopez & Lindsay, 1993; Kilic & Lindsay, 2005). Qualitative and quantitative differences in the profile of phenolic compounds of milk from different ruminant species have been reported. Phenols in sheep milk were mostly bound as phosphate and sulfate conjugates while in cows' and goats' milk they were mainly bound as sulfates. Levels of *p*-cresol and *m*-cresol were higher in sheep than in goats' and cows' milk but goats' milk contained an exceptionally high concentration of phenol. These alkyl phenols were present as glucuronide and sulfate acid conjugates in all milks (Lopez & Lindsay, 1993). Most phenolic compounds derive from the feed but other possible sources are from amino acid catabolism by bacteria and contamination with

sanitizing agents (O'Connell & Fox, 2001). Although very little is known on the effect of diet on the content of non-volatile phenolic substances in milk, recent research has shown the accumulation of phenolic compounds in the milk of grazing goats (Silanikove *et al*., 2010). At low levels, phenolic compounds may impart desirable sweet, smoky, or caramel notes, but at high levels they can cause off-flavors such as sharp or medicinal (O'Connell & Fox, 2001).

15.3.2 Variations in flavor of fresh milk from ruminant species

Milk is a natural food, biologically produced and, therefore, changes in its flavor are expected (Thomas, 1981). In raw milk, these changes are mainly related to the dairy cattle management system (genetic, physiology, and feeding) and environmental hygiene conditions at the farm level. Variations in milk flavor seem to be caused by concentration differences of a common set of volatile compounds rather than by the occurrence of certain compounds uniquely associated with a particular feed (Bendall, 2001; Mounchili *et al*., 2005). Milking, collection, and storage of raw milk on farms, processing and storage in dairy industries, and displaying and stocking in markets can also modify the characteristic volatile profile of fresh milk.

Aspects related to milk flavor variability are of enormous relevance for the industry as they can lead to serious defects in dairy products. For this reason, the main sources of variations in fresh milk flavor are discussed below.

15.3.2.1 Variations in milk flavor associated with farm management

It is generally agreed that the manufacture of high-quality dairy products begins at the farm level. Milk quality is affected by genetic and physiological characteristics of the ruminants, and the milking systems, storage, and collection of the milk, hygienic conditions of the milking equipment, the farm workers, and the farm overall (McGilliard & Freeman, 1972; Manfredini & Massari, 1989; Coulon *et al*., 2004). Also diets and feeding systems chosen and practiced by the farmer have been shown to influence the sensory quality of milk (Morand-Fehr *et al*., 2007; Butler *et al*., 2011; Zervas & Tsiplakou, 2011). In particular, small ruminant milk flavor is highly influenced by season, diet, milking and cooling practices, and the barn environment (Moio *et al*., 1996; Carunchia Whetstine & Drake, 2006).

15.3.2.1.1 Genetic and physiological characteristics

Breed and other genetic traits of the milking animals, besides age, health status, and lactation stage have important influences on milk flavor. Early studies reported that genetic differences and lactation stage were important sources of variations in milk flavor, whereas the cow's age at calving had little effect on flavor characteristics (Kratzer *et al*., 1967; McGilliard & Freeman, 1972). Studies conducted on goats' milk have indicated that a strong goat flavor can be attributed to milk produced in the middle of lactation with low fat content, high somatic cells counts, and free fatty acid levels compared with herd milk with weak goat flavor intensity (Skjevdal, 1979; Jaubert *et al*., 1996).

15.3.2.1.2 Feeding systems and diets

There is a wide range of feeding systems all over the world and they can be grouped in different types. The two major systems are pasture and indoor feeding, which have variable levels of intensification from very extensive to very intensive (Zervas & Tsiplakou, 2011). Feed types (such as forages, concentrates, by-products), forage preservation methods (hay or silage), pasture grass botanical composition, and different types of diets have been extensively studied as a medium to increase either the unsaturated fatty acid levels (especially conjugated linoleic acid, CLA) or the antioxidative capacity of milk. In studies of the relationship between diets given to ruminants and free fatty acid composition of milk fat it has been shown that a high content of unsaturated fatty acids in milk fat may not necessarily be positive for milk quality since the double bonds of fatty acids can promote defects, mainly via oxidative reactions. For this reason, the oxidative stability of milk is still a topic of concern to the dairy industry. Among several implications for milk quality, a shorter shelf-life, off-flavor development (e.g., oxidized, grassy and cowy), and nutritional deterioration can occur (Havemose *et al*., 2004, 2006). Also, specific off-flavors in milk have been attributed to specific diet components, and the compounds responsible have been identified (Urbach, 1990a).

Defects produced by free fatty acid oxidation

The oxidative stability of milk results from a delicate balance between antioxidant and pro-oxidant activities, influenced in turn by several factors such as composition of fatty acids, transition metal ions, and antioxidants (Smet *et al*., 2008). Manipulation of feeding can increase the content of unsaturated fatty acids and make the milk more susceptible to oxidation, or improve the oxidative stability of milk if antioxidants can be transferred from feed to milk (Havemose *et al*., 2004). Numerous studies have focused on sensory characteristics of milk naturally enriched with polyunsaturated fatty acids, mainly CLA. It has been suggested that pasture-based (PB) production systems may contribute to health benefits for the consumer due to the presence of higher concentrations of CLA than when cows were fed concentrates, silages, or conventional total mixed

rations (TMRs) (White *et al*., 2001; Elgersma *et al*., 2006; Butler *et al*., 2011). Milk obtained from PB and TMR systems showed some differences in the volatile profile and overall flavor (Bendall, 2001; Croissant *et al*., 2007), but whereas trained panelists reported greater intensities of grassy and cowy/barny flavors in PB milk than in TMR milk, consumers were unable to differentiate them (Croissant *et al*., 2007). Other studies have considered the effects of diets with different ratios of red clover and grass silage on the volatile profile or sensory characteristics of milk, since the incorporation of red clover silage leads to increased levels of polyunsaturated fatty acids. Results obtained so far are contradictory. Al-Mabruk *et al*. (2004) reported a decrease in oxidative stability during the storage of milk produced from red clover silage compared with milk from diets based on grass silage. According to Moorby *et al*. (2009) there were no effects on the aroma or overall flavor of milk when the proportion of red clover in the diet of dairy cows was increased. Oxidative stability of milk enriched with natural CLA through diets supplemented with soybean oil and fish oil has been also evaluated. Lynch *et al*. (2005) analyzed both flavor and stability of pasteurized milk with and without naturally increased levels of CLA. Untrained panelists were unable to detect flavor differences during the storage period and, thus, sensory analysis indicated no difference in susceptibility to the development of oxidized off-flavors between both types of milks. Most research carried out on sensory characteristics of milk enriched with CLA has obtained the same results (Baer *et al*., 2001; Ramaswamy *et al*., 2001a, b; Nelson & Martini, 2009), which suggests that the levels of increased CLA do not promote the oxidative deterioration of milk.

The effect of antioxidants and pro-oxidants on oxidative stability of milk has also been evaluated, since it was suggested that oxidized off-flavor could be inhibited by the presence of certain antioxidants such as α-tocopherol, β-carotene, ascorbic acid, and selenium or enhanced by the action of certain pro-oxidants such as copper (Barrefors *et al*., 1995; van Aardt *et al*., 2005a; Havemose *et al*., 2006). Milk with high unsaturated fatty acid levels is most susceptible to oxidation, especially when it has high concentrations of copper (Timmons *et al*., 2001). Natural (tocopherols, ascorbic acid, etc.) and synthetic (butylated hydroxyanisole, butylated hydroxytoluene, etc.) antioxidants have attracted much attention as a medium of preventing or delaying oxidation. Milk contains low concentrations of natural antioxidants, which also depend on feeding, but they can only initially avoid oxidative deterioration (van Aardt *et al*., 2005a). The effect of antioxidants on the oxidative stability is highly dependent on their concentrations and on the level of polyunsaturated fatty acids in milk. For similar concentrations of natural antioxidants, raw milk containing more unsaturated fatty acids was more sensitive to oxidation (Hedegaard *et al*., 2006). According to Havemose *et al*. (2004, 2006), the role of natural antioxidants to prevent lipid oxidation seems to be less important than the fatty acid composition. By contrast, milk supplementation with natural or synthetic antioxidants was effective against oxidized off-flavor during storage (Al-Mabruk *et al*., 2004; van Aardt *et al*., 2005a, b). Recently, oxidative stability of milk was evaluated in relation to selenium supplementation to the cow's diet (Clausen *et al*., 2010). The results indicated that this natural antioxidant has no effect on oxidative stability of milk.

Among the tools available to determine the oxidative stability of milk as a function of time, the measure of certain primary and secondary oxidation products might to be a good parameter (Hedegaard *et al*., 2006). Oxidative off-flavor is closely related to increased concentrations of saturated straight-chain aldehydes from C5 to C9 (Jenq *et al*., 1988; Barrefors *et al*., 1995). In particular, the assessment of hexanal correlate well with oxidized sensory descriptors (Hedegaard *et al*., 2006). Similarly, sulfur-containing compounds (dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide), alkenals (2-octenal, 2-heptenal, 2-nonenal), alkadienals (2,4-nonadienal, 2,4-decadienal, etc.), unsaturated ketones (1-octen-3-one), and alcohols (1-octen-3-ol) have also been reported as typical compounds that impart oxidized off-flavors to dairy products (Barrefors *et al*., 1995; van Aardt *et al*., 2005a, b; Marsili, 2011).

Defects related to specific feeds

Feeding of the dairy herd can affect not only fatty acid composition of milk fat and oxidative stability of milk. Certain diets and grass botanical composition can affect the volatile profile by modifying the quantities of some compounds that belong to chemical groups such as terpenes (Mariaca *et al*., 1997; Bugaud *et al*., 2001; Addis *et al*., 2006; Tornambé *et al*., 2006), hydrocarbons (Addis *et al*., 2006), aldehydes (Moio *et al*., 1996; Bendall, 2001; Toso *et al*., 2002), lactones (Bendall, 2001), sulfur and nitrogen compounds (Moio *et al*., 1996; Bendall, 2001), and phenolic compounds (Silanikove *et al*., 2010), which not necessarily lead to reduced sensory quality of milk. However, certain fodder plants and silages consumed by cows such as fermented musty silage (maize, legumes, and grass), sugar beet by-products, fruit and vegetable residues, alfalfa (green or hay), clover hay, onion weeds, and green barley have been linked to negative sensory attributes, giving milk some undesirable notes such as fishy, onion, silage, herb-like, green grass (Marsili, 2011). This topic has been extensively reviewed by Urbach (1990a) and therefore only some examples are mentioned. Trimethylamine

and methyl sulfide were largely reported as responsible for the fishy and malty/cowy flavors of milks from cows fed wheat pasture and freshly cut alfalfa, respectively (Reddy *et al*., 1966; Mehta *et al*., 1974). A specific rancid and tart taste, probably due to lipolysis of milk fat, is the most common defect in Norwegian goats' milk during some seasons, whose frequency has been related to certain types and levels of concentrates and roughage (Eknaes & Skeie, 2006; Eknaes *et al*., 2009). Silages have been specifically investigated as a potential source of feedy or silage notes. Silages that are not well fermented are most commonly responsible for imparting this adverse taste to milk. The term "feedy" is used to describe a variety of flavors having similar characteristics. In early studies it was reported that acetone, 2-butanone, dimethyl sulfide and *cis*-3-hexen-1-ol caused tainted milk by feedy flavors (Shipe *et al*., 1962). Recently, 2-butanone and dimethyl sulfide (and to some extent ethanol and 2-propanone) were suggested as probable markers for this defect (Mounchili *et al*., 2005).

15.3.2.1.3 Environmental conditions

Studies on the causes of flavor changes in goat milk have shown that unclean handling of the milk reduces the genuine flavor (Skjevdal, 1979). In an atmosphere dominated by silage or animal odors, volatile compounds may be transferred directly from the surrounding environment to the milk. Transmitted or absorbed off-flavors can occur in milk before, during, and after milking. The odor of the male goat in rut is often implicated as a source of the goaty flavor problem in fresh goats' milk. The high volatility of buck odors has led to a recommended practice on farms of isolating the buck some distance from the milking area; 6-*trans*-nonenal generated by the sebaceous gland of the scalps of sexually active bucks as a pheromone or as a product from oxidation of certain precursors in the gland lipids was identified as a major contributor to this odor (Smith *et al*., 1984).

15.3.2.1.4 Raw milk quality and milk manipulation

The main causes of flavor variations in raw milk quality are from milk manipulation related to the activity of enzymes in the milk, spoilage microorganisms or somatic cells, and off-flavors produced by lipolysis and proteolysis are the most common.

Defects caused by induced and spontaneous lipolysis

Hydrolytic rancidity of milk lipids or lipolysis is perhaps the main defect observed in farm milk. This defect must be distinguished from the oxidative rancidity (Deeth, 2011) resulting from lipid oxidation. The hydrolysis of triglycerides, the major constituents of milk fat, is catalyzed by lipases producing free fatty acids, some of which have low perception thresholds and unpleasant aromatic notes (rancid, butyric, bitter, unclean, soapy, or astringent) (Deeth & Fitz-Gerald, 2006; Deeth, 2011). Endogenous milk lipase or lipoprotein lipase (LPL) is one of the main types of enzymes responsible for this reaction. The natural lipolytic system and fatty acid composition of milk fat differ considerably between ruminant species (Chilliard *et al*., 2003). Lipase activity in ovine milk is about one-tenth and in goats' milk is about one-third than that of bovine milk. Goats' milk fat has significantly higher levels of short- and medium-chain linear fatty acids (C4:0 to C14:0) and branched-chain fatty acids than cows' milk (Park 2006). Owing to the specificity of the LPL, the hydrolysis patterns of milk fat are also different (Albenzio & Santillo, 2011). It has been reported that linear short-chain fatty acids contribute markedly to rancid off-flavors in bovine milk (Scanlan *et al*., 1965; González-Córdova & Vallejo-Cordoba, 2001), medium-chain fatty acids can be responsible for this defect in ovine milk (Albenzio & Santillo, 2011), whereas in goats' milk rancidity is due to free fatty acids from C6:0 to C9:0 and more specifically from volatile branched-chain acids such as 4-methyl and 4-ethyl octanoic acids (Eknaes *et al*., 2009).

In raw milk, little or no lipolysis should occur, as the lipase usually cannot make contact with the substrate, since the triacylglycerols are protected in globules covered by a membrane (Escobar & Bradley, 1990). Rancidity is caused by weakened or broken milk fat globule membranes, which is promoted by several factors. Two types of lipolysis seem be related to the action of native lipases of milk of good milk quality: induced and spontaneous.

Induced lipolysis is related to the damage of the globule membrane caused by pumping, mechanical agitation, foaming, mixing of air into the milk, milking systems, temperature changes, slow cooling, freezing and thawing in storage (Escobar & Bradley, 1990; Slaghuis *et al*., 2004; Deeth, 2011). Certain management practices on the farm, including the use of modern but improperly designed milking systems, can induce rancidity. Automatic robot milking systems can lead to milk with higher free fatty acid levels than that obtained in a conventional milking parlor (Abeni *et al*., 2005). Inadequate maintenance, faulty design and installation of milking machines, teat cups, and pipelines can produce excessive air intake with the milk, causing turbulence and frothing of the milk, and thus damage to the fat globule membrane (Slaghuis *et al*., 2004; Deeth, 2011). High pipeline milking systems, lifting milk by air in vertical pipe sections, especially with a high air to milk flow ratio, and high speed of pumping of milk to the bulk tank are some design features that promote increased levels of lipolysis (Escobar & Bradley, 1990; Slaghuis *et al*., 2004).

Spontaneous lipolysis is defined as that which occurs in some individual milk when cooled soon after milking without any other treatment. Individual cows differ in the susceptibility of their milk to spontaneous lipolysis (Escobar & Bradley, 1990; Deeth & Fitz-Gerald, 2006). This phenomenon is observed most often when a large number of cows are in late lactation and/or when good-quality feed is not available (Deeth, 2011). In this case, the fat globule is probably not disrupted, but several factors favor the interaction of LPL with milk fat (Slaghuis *et al*., 2004). Some management practices on farms such as milking frequencies, generally associated with automatic milking, apparently lead to increased free fatty acid levels (Abeni *et al*., 2005). However, one of the main factors influencing spontaneous lipolysis is the activator/inhibitor ratio (Cartier & Chilliard, 1990). In bovine milk, lipolysis remains generally low despite the high LPL activity. This could be due to inhibitors and to the fact that in bovine milk LPL is largely bound to casein micelles, compartmentalized from the substrate. In contrast, large proportions of LPL are bound to cream in goat milk, which could explain its higher sensitivity to spontaneous lipolysis. In goat milk the lipolysis level correlates well to LPL activity (Chilliard *et al*., 2003; Park, 2006).

Defects caused by microorganism growth

Psychrotrophic bacteria are the dominant type of bacterial flora in raw milk after a period of refrigerated storage (Deeth, 2011). They multiply at low temperatures and may have negative effects to lipolysis and proteolysis if the storage is prolonged (Manfredini & Massari, 1989; Celestino *et al*., 1996). Rancid, unclean, soapy, cardboard, oxidized, and metallic flavors are typical defects produced by lipases of psychrotrophic bacteria via lipolysis (Champagne *et al*., 1994). In addition, putrid flavor caused by proteolysis is the result of bacterial contamination, holding raw milk for 3 or 4 days after collection, and storage temperature above 5°C (40°F) (Dairy Practices Council, 1991).

Defects caused by high somatic cell counts

Another important source of flavor variation, closely linked to the bacteriological quality of the milk, is the somatic cell count (SCC). SCC is dependent on ruminant species and dairy herd management (breed, stage of lactation, season, udder health, etc.) (Albenzio & Santillo, 2011), and it is considered a good index of the hygienic and sanitary aspects of the milk (Raynal-Ljutovac *et al*., 2007). Both bacterial load and SCC can be diminished by improved management conditions, which include sanitation at the farm of animals and milking facility, udder cleaning and teat sealing, milking equipment maintenance, and timely transfer to the refrigerated tank (Goetsch *et al*., 2011). Increased SCC is correlated with increased levels of proteolysis and lipolysis and a higher activity of heat-stable protease (plasmin) and lipase (LPL) enzymes even during refrigerated storage of raw and pasteurized milk (Senyk *et al*., 1985; Ma *et al*., 2000; Barbano *et al*., 2006; Gargouri *et al*., 2008). Main sensory defects related to high SCC include bitterness and rancidity (Ma *et al*., 2000). Hydrophobic peptides such as those derived from β-casein are related to the appearance of bitterness (Harwalkar *et al*., 1993), while lipolysis causes rancid off-flavor (Raynal-Ljutovac *et al*., 2007). Bovine milk with high SCC, pasteurized and stored refrigerated, developed rancidity as the predominant flavor defect between 14 and 21 days (Ma *et al*., 2000), but proteolysis may produce off-flavor earlier during shelf-life than lipolysis (Barbano *et al*., 2006). Concerning small ruminant milk, increased goaty flavor and lipolytic activity were partly associated with higher levels of SCC (Jaubert *et al*., 1996). Proteolysis, plasmin activity and SCC were positively correlated in goats' and ewes' milk (Raynal-Ljutovac *et al*., 2007).

Lipolysis and proteolysis thresholds

Off-flavors caused by hydrolysis of triglycerides and of proteins by proteases or lipases from somatic cells, endogenous in milk or of bacterial origin, are severe drawbacks for the quality of dairy products and therefore their sensory thresholds have been assessed. Traditionally, the acid degree value (ADV) has been used as a measure of hydrolytic rancidity (González-Córdova & Vallejo-Cordoba, 2001). According to this index, the threshold for lipolyzed flavor detection in milk is within the range 4.1–4.5 mEq KOH/100 g fat (Pillay *et al*., 1980), or approximately 1.0 mEq FFA/100 g fat (Ma *et al*., 2000). More recently, the sensory threshold for lipolysis in milk with a fat content of 2% was determined by an ascending forced-choice procedure, with a series of triangle tests in different sessions. In this study, the lipolysis detection threshold was established between 0.32 and 0.35 mEq of free fatty acid per kilogram of milk (Santos *et al*., 2003), whereas the sensory threshold of bitterness by proteolysis in milk with 2% fat and skim milk was equivalent to a decrease of 4% and 4.8% of the casein, respectively (Ma *et al*., 2000; Santos *et al*., 2003).

15.3.2.2 Variations in milk flavor associated with factory management

Milk industrialization influences the natural fresh milk flavor. Depending on the industrial practices, some changes can be detrimental. Among the potential sources of off-flavor are manipulation and storage of raw milk, severity of thermal treatment and storage conditions of processed milk including packaging.

15.3.2.2.1 Manipulation and storage of raw milk

Owing to changes in milk collection from farms and management practices at dairies, some fluid milk plants may be processing raw milk as old as 5 days (Champagne *et al*., 1994). In dairy plants, certain conditions that promote lipolysis and psychrotrophic bacteria growth can take place. These are failures to empty and wash raw milk from tanks, air leaks in pipes, excessive pumping, homogenization without immediate pasteurization, and mixing of homogenized and raw milk (Dairy Practices Council, 1991; Deeth, 2011).

Defects caused by lipolysis in pasteurized milk are the same as those produced in raw milk. The dominant species limiting the shelf-life of refrigerated fluid milk is *Pseudomonas* spp. (Dogan & Boor, 2003). Psychrotrophs are destroyed by thermal treatments but their enzymes are thermostable. In this case, *Pseudomonas* spp. need to grow at relatively high levels in raw milk before pasteurization to produce verifiable defects in processed milk at the end of shelf-life (Barbano *et al*., 2006). Furthermore, they may contaminate milk after pasteurization if the pumping, holding, and filling system is not properly cleaned and sanitized.

Undesirable flavors due to microbial growth include bitter, rancid, fruity, and unclean (Champagne *et al*., 1994). *Pseudomonas fragi* is recognized as the microorganism responsible for the production of a fruity or strawberry-like odor, with ethyl butanoate, ethyl hexanoate and ethyl-3-methyl butanoate the major contributors (Cormier *et al*., 1991). Another microbial-induced off-flavor is a malty aroma due to methyl aldehydes and methyl alcohols such as 2-methylbutanal, 3-methylbutanal, 2-methyl-1-butanol, and 3-methyl-1-butanol (Marsili, 2011). *Lactococcus lactis* var. *maltigenes* is the main microorganism responsible for this defect, which is related to poor refrigeration of milk.

15.3.2.2.2 Severity of thermal treatment

More rigorous thermal treatments than that strictly needed for pasteurization can be an effective approach to extend milk shelf-life, and is applied on many occasions, depending on the dairy plant and the country. However, severe heat treatment can cause negative sensory attributes and consequently a decrease in the acceptability of milk by consumers. This topic was recently investigated by Gandy *et al*. (2008), who analyzed the effect of four pasteurization temperatures (77, 79, 82, and 85°C/15 s) on consumer acceptability, sensory characteristics, and shelf-life of fluid milk. This study revealed that milk processed up to 79°C was highly acceptable to all consumers. This research also showed that milk samples could not be differentiated based on pasteurization temperature when tested toward the end of shelf-life, suggesting that sensory differences evened out as storage time elapsed.

15.3.2.2.3 Storage conditions of processed milk and packaging type

Milk flavor can be strongly altered as a consequence of the storage conditions. The packaging material determines largely the degree to which certain physical agents can act, promoting undesirable reactions (Moyssiadi *et al*., 2004). An undesirable flavor known as packaging can be induced by contact of milk with certain types of packaging materials.

Packaging material can maintain the pasteurized milk quality by controlling oxygen permeability and light transmission as well as by providing perfect seals to avoid microbial recontaminations (Vassila *et al*., 2002). In addition to the traditional glass bottles and coated paperboard cartons, several plastic containers, such as polyethylene terephthalate (PET) and high-density polyethylene (HDPE) available as clear, pigmented, monolayer, and multilayer bottles, are commonly used in pasteurized milk packaging. Moreover, polyethylene pouches in the form of flexible monolayer or low-density polyethylene (LDPE) and multilayer coextruded pouches based on LDPE are also found in the market (Vassila *et al*., 2002; Zygoura *et al*., 2004).

Oxidation in processed dairy products is due to acceleration of reactions already initiated in the raw milk (Hedegaard *et al*., 2006). The two main mechanisms of milk oxidation are the oxidation induced by certain metals or autoxidation, clearly prevalent in light-protected milk, and oxidation induced by light (Smet *et al*., 2008). Different patterns of flavor deterioration are observed in each case (Karatapanis *et al*., 2006).

Light-oxidized off-flavor can develop when fresh milk is packaged in light-transmissible containers and then stored in lighted displays (Chapman *et al*., 2002). It has been estimated that exposure to light of milk packaged in HDPE containers and exposed in dairy cabinets is responsible for light-induced flavor defects in 80% of samples sold in supermarkets in USA (Marsili, 2011). Light exposure can also adversely affect the nutritional value of milk producing vitamin loss (Olsen & Ashoor, 1987; Lee *et al*., 1998; Whited *et al*., 2002). Both light and riboflavin participate in photo-induced chemical reactions that involve certain fatty acids and amino acids as substrates (Kim & Morr, 1996). Typical descriptors related to the oxidation processes include old vegetable oil, cardboard, goat, or metallic (Chapman *et al*., 2002; Hedegaard *et al*., 2006). The presence of methional, an initial product of methionine degradation, is directly related to exposure of milk to light (Allen & Parks, 1975). Other volatile compounds such as methanethiol, dimethyl sulfide, dimethyl disulfide, pentanal, hexanal, 2-butanone, and 1-octen-3-one have been also identified as light-activated flavors (Jenq *et al*., 1988; Kim & Morr, 1996; Karatapanis *et al*., 2006; Marsili, 2011). This defect is rapidly perceived by trained panelist

and consumers. Sensory thresholds of light-oxidized off-flavors were determined by Chapman *et al*. (2001). According to these authors trained panelist were able to detect defects in reduced fat milk packaged in HDPE containers after 15–30 min of exposure to fluorescent light while consumers detected light-induced off-flavors after 54 min to 2 hours.

Numerous studies have compared the effect of several packaging materials on keeping quality (defined as the number of days between manufacture and spoilage) of pasteurized milk by determining lipid oxidation, induced-light oxidation, vitamin degradation, proteolysis, lipolysis, and microbial growth. Bottles made from PET incorporating UV blocking agents (Cladman *et al*., 1998; van Aardt *et al*., 2001; Papachristou *et al*., 2006) and multilayer pigmented HDPE (Moyssiadi *et al*., 2004; Zygoura *et al*., 2004; Karatapanis *et al*., 2006), coextruded pouches based on LDPE containing both white and black pigments and thickness above 100 μm (Vassila *et al*., 2002) and laminated cartons with ethylene-vinyl alcohol and aluminum foil (Simon & Hansen, 2001b) have been reported as packaging that provides the best overall protection for the pasteurized milk, being attractive and convenient alternatives to the coated paperboard cartons.

As for the packaging off-flavor, its presence was detected in milk induced by contact with PE film and PE-coated paper. The defect is easier to perceive in skim milk, and container and the area/volume ratio of the package is important for its development, while the type of heat-sealing does not appear to influence it. The off-flavor appears at the beginning of the storage and does not increase with time (Leong *et al*., 1992).

15.3.3 Volatile profile and sensory characteristics of heat-treated milk

Thermal processes alter the sensory characteristics of milk. The kind and intensity of this change is related to the time and temperature of the treatment (Scanlan *et al*., 1968; Shipe *et al*., 1978). The appearance of heat-induced flavors is inevitable in any heated milk, but mainly in those treated with more rigorous temperature–time conditions, such as UP, UHT processes, concentration, in-container sterilization, and drying than conventional pasteurization. As the intensity of thermal treatment increases, the levels of volatile compounds derived from proteins, carbohydrates, and lipids also are augmented, and the heat-induced flavors are more strongly detected (Badings & Neeter, 1980; Calvo & de la Hoz, 1992). Thus, the typical flavor of fresh UHT milk is described as cooked or cabbagey, whereas flavor of sterilized and concentrated milk is characterized by caramelized or burnt notes (Nursten, 1997). Certain indexes can give information about the thermal history of a milk sample, namely the degree of whey protein denaturation and the presence of compounds from Maillard reactions (Guingamp *et al*., 1999) or the levels of a reduced set of volatile compounds thermally formed (Contarini *et al*., 1997).

15.3.3.1 Ultrapasteurized milk and ultra-high-temperature treated milk

The sensory characteristics of UP milk must be similar to those of pasteurized milk (Simon & Hansen, 2001a) and therefore attributes of heated milk should be slightly perceived. Chapman *et al*. (2001) described the key sensory characteristics of UP milk samples of different fat levels using quantitative descriptive analysis. Intensity of descriptors was higher for cooked, sweet, and caramelized notes, indicating their relevance in overall flavor. Flavor development in milk of extended shelf-life is a dynamic process that evolves over time. Cooked notes that characterize fresh UHT and UP milks dissipate after a few days depending on storage temperature (Celestino *et al*., 1997b; Chapman *et al*., 2001), possibly due to loss of volatile sulfides (Simon *et al*., 2001) or by oxidation of sulfhydryl groups (Celestino *et al*., 1997b; Simon & Hansen, 2001a), giving a maximum acceptability after a few days (Nursten, 1997). Then, in a second stage, flavor of UHT or UP milk suffers deterioration and the overall quality declines slowly during storage as milk develops a flavor described as stale, bitter, heated, or sterile (Nursten, 1997; Chapman *et al*., 2001). It has been reported that a slight sweet taste can appear after the cooked flavor has dissipated, which is commonly considered as acceptable (Celestino *et al*., 1997b).

Cooked and cabbagey notes have been attributed to volatile sulfur compounds such as hydrogen sulfide, methanethiol, methyl disulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl sulfone, among others (Al-Attabi *et al*., 2008). As the processing temperature increases, the levels of these compounds increase in parallel (Simon *et al*., 2001; Vazquez-Landaverde *et al*., 2005). The main source of them is the sulfhydryl group of proteins, mainly whey proteins such as β-lactoglobulin and to a minor extent fat globule membrane proteins, which can react during heating (Clare *et al*., 2005). An interesting research about kinetic modeling of the formation of sulfur-containing components during heat-treatment of milk was performed by de Wit & Nieuwenhuijse (2008). According to this study, cooked flavor showed high positive correlations with the concentration of methanethiol.

In spite of its importance on overall flavor, sulfur compounds are not the main volatile components of heated milk from a quantitative viewpoint. Methyl ketones have been reported as the most abundant class of compounds (Jeon *et al*., 1978; Contarini *et al*., 1997; Valero *et al*., 2001; Solano-Lopez *et al*., 2005), and it is well known that

they are produced during thermal treatments (Contarini *et al*., 1997; Vazquez-Landaverde *et al*., 2005). Although a minor role on milk flavor has been reported (Jeon *et al*., 1978), methyl ketones have been extensively studied since their content is related to the intensity of thermal treatment applied to milk (Scanlan *et al*., 1968; Contarini *et al*., 1997). In particular, the concentration of 2-heptanone seems to be a suitable marker for heat treatment (Contarini & Povolo, 2002; Avalli *et al*., 2004).

Aldehydes, lactones, alcohols, nitrogen-containing compounds, esters, and hydrocarbons are other classes of volatile compounds commonly identified in UHT and UP milks (Jeon *et al*., 1978; Simon *et al*., 2001; Valero *et al*., 2001; Solano-Lopez *et al*., 2005). Among them, benzothiazole, δ-lactones, and hexanal have been suggested as typical heat-induced compounds (Scanlan *et al*., 1968; Calvo & de la Hoz, 1992; Contarini *et al*., 1997), making a moderate contribution to the flavor of this type of milk (Moio *et al*., 1994). Unlike in fresh milk, esters have a secondary role on flavor, which has been attributed to their thermal destruction (Moio *et al*., 1994; Marsili, 2011).

15.3.3.2 Milk powder, sterilized, and concentrated milk

Among thermally treated milks, a special consideration has always been given to milk powder or dry milk. Ideally, milk powder should have aromatic notes similar to fluid milk, that is, cooked, sweet, and milk fat free of defects such as grassy or painty flavors (Lloyd *et al*., 2009a). Skim milk powder (SMP) and whole milk powder (WMP) are widely used as food ingredients and for direct consumption (Karagül-Yüceer *et al*., 2001). However, these two types of milk powders have some flavor differences. Studies carried out by Drake *et al*. (2003) on sensory attributes of milk powder have indicated that cooked, sweet aromatic, salty, astringent, cardboard, potato/brothy, cereal, and animal are attributes frequently observed in SMP. Cooked flavors may be present and vary according to heat treatment (low, medium, and heat) of the milk prior to evaporation. The application of principal component analysis to characterize and to distinguish milk powder samples according to their attributes showed some interesting results. SMP samples were grouped into three categories. One group was characterized by sweet aromatic, sweet taste, and cooked, being the most closely associated with the typical flavor of fluid skim milk. Another group was characterized by animal, potato/brothy, and astringent flavor and a few samples were described by cereal/grassy and cardboard notes. On the other hand, attributes reported for WMP were described as cooked, caramelized, sweet aromatic, milk fat, fried, salty, astringent, cereal, among others. Certain flavors such as milk fat, fried and fatty/painty were only detected in WMP, which is in accordance with the fact that these flavors are derived from milk fat.

Volatile compounds present in fresh milk powder have been extensively investigated (Shiratsuchi *et al*., 1994a; Karagül-Yüceer *et al*., 2001, 2002; Lloyd *et al*., 2009a, b). Key aroma compounds identified in SMP were verified by sensory analysis on model mixtures (Karagül-Yüceer *et al*., 2004). From these studies the important contributions were established to the overall flavor for free fatty acids, lactones, and a set of heterocyclic compounds such as furaneol, maltol, and sotolon, and the minor role of hydrocarbons, saturated and non-saturated aldehydes, methyl ketones, and alcohols. The chemical nature of these compounds reveals the key role of milk fat hydrolysis and Maillard reactions, which are chemical processes encouraged by heat. Sweet and milk odors in SMP have been correlated with levels of nonanoic, decanoic, undecanoic, and dodecanoic acids and some δ- and γ-lactones (Shiratsuchi *et al*., 1995).

Flavor of sterilized concentrated milk has been described as caramelized, which intensifies during storage at room temperature. Analysis of volatile compounds has revealed the presence of methyl ketones, benzaldehyde, *o*-aminoacetophenone, 2-furfural, furfuryl alcohol, hydroxymethylfurfural, benzothiazole, fatty acids, and δ-lactones (Arnold & Lindsay, 1969; Loney & Bassette, 1971; Badings & Neeter, 1980). Among them, methyl ketones could contribute to overall flavor (Allen & Parks, 1969; Arnold & Lindsay, 1969). Taking into account that the levels of some of these compounds such as methyl ketones, *o*-aminoacetophenone and free fatty acids may increase during storage and thus generate off-flavor, their evolution through the storage process has also been investigated (Patel *et al*., 1962; Loney & Bassette, 1971).

15.3.3.3 Infant formula

They are substitutes of human milk for infants when a mother cannot breastfeed or chooses not to breastfeed (O'Callaghan *et al*., 2011). Since it was largely suggested that breast milk has a higher acceptance by infants than formulated milks and both seem to differ in flavor, attention has been focused on differences in volatile composition and sensory characteristics between them. Hausner *et al*. (2009) found that the volatile fraction of human breast milk was characterized by aldehydes, terpenes, alcohols, and ketones, whereas carbonyl compounds such as ketones and aldehydes represented the most important class of volatiles in infant formula milk. According to these authors, formulae differed from mothers' milk as they contained some volatiles related to heat treatment such as methional, 2-furfural and sulfides, which were not present in mothers' milk. On the other hand, breast milk had a higher variety of terpenes, aldehydes, and alcohols.

15.3.4 Variations in flavor of heat-treated milk

Heating affects milk quality as a consequence of interactions between amino acid lateral groups, degradation reactions of lateral chains of the proteins, restructuring of SH and S–S groups, whey protein insolubilization, interactions between κ-casein and β-lactoglobulin, interactions of proteins with lipids, and interactions between carbohydrates and proteins, namely the Maillard reaction (Ferrer *et al*., 2000a). From a sensorial viewpoint, the potential sources of undesirable flavors are commonly related to lipid oxidation and Maillard reactions. Flavor defects may also indicate spoilage and microbial growth.

15.3.4.1 Ultrapasteurized milk and ultra-high-temperature treated milk

Oxidized, cardboardy, lack of freshness, fruity, and hammy are the most common off-flavors reported in UP milk (Simon & Hansen, 2001a; Simon *et al*., 2001). Chapman *et al*. (2001) observed that bitter flavor, bitter aftertaste, drying, and lingering aftertaste develop in UP samples at the end of shelf-life, contributing to the reduction in the overall quality rating. These defects were attributed to the enzymatic activity of lipases and proteases that can survive thermal processes. Flavor deterioration in UP milk has not been investigated as extensively as in UHT milk and the few existing research studies have focused on the effects of processing temperature and packaging material on shelf-life and flavor (Simon & Hansen, 2001a; Simon *et al*., 2001; Solano-Lopez *et al*., 2005). Results showed that milk processed at higher temperatures contained the highest relative amounts of sulfur compounds, whose retention in the product depended on the type of barriers and foil boards of the packaging. Hammy, cardboardy, and cooked notes derived from packaging type and their intensity increased with storage time, so that rates of milk flavor deterioration varied according to packaging materials. Rancidity was not reported as a problem during storage of UP milk.

Cooked note in UP milk is minimal compared with regularly processed UHT milk, which can develop heat-induced off-flavors (Solano-Lopez *et al*., 2005). Certain thermally derived compounds such as 2,3-butanedione, 2-heptanone, 2-nonanone, 2-methylpropanal, 3-methylbutanal, nonanal, decanal, and dimethyl sulfide were identified as important contributors to the off-flavor of UHT milk (Vazquez-Landaverde *et al*., 2005). In addition to defects induced by heat, other causes of low sensory quality of stored UHT milk seem to be proteolysis, lipolysis, oxidative reactions, and non-enzymatic browning (Al-Kanhal *et al*., 1994; Valero *et al*., 2001; Gaucher *et al*., 2008).

Enzymatic processes occur as a consequence of the reactivation of heat-resistant enzymes during prolonged storage (Chen *et al*., 2003). Although both proteolysis and lipolysis are considered the most important factors limiting the shelf-life of UHT milk during storage at room temperature through changes in flavor and texture, the main enzymatic defects reported are related to proteolysis. Bitter, unclean, sour, and putrid notes are attributed to the action of plasmin and/or proteinases from spoilage microorganisms of raw milk (Datta & Deeth, 2003). Recommendations on the limits of proteinase activity or tyrosine levels in UHT milks to ensure certain shelf-life have been proposed, but these values are highly dependent on assay techniques (Chen *et al*., 2003).

Stale, metallic, and/or oxidized off-flavor derived from lipid oxidation can be detected during storage of UHT milk with different intensity (Valero *et al*., 2001). Cooked flavors mask or prevent the detection of oxidative defects at the beginning of storage, but as sulfhydryl groups are oxidized and volatile sulfur compounds dissipate, defects can be perceived (Celestino *et al*., 1997b). Concentrations of certain carbonyl compounds, mainly straight-chain aldehydes and methyl ketones have been closely related to staleness (Jeon *et al*., 1978; Rerkrai *et al*., 1987).The contribution of dissolved oxygen, packaging type and storage conditions (time and temperature) on its development has been investigated. In general, staleness is more pronounced at higher temperatures and longer times of storage (Mehta & Bassette, 1980; Al-Kanhal *et al*., 1994; Celestino *et al*., 1997b). The role of oxygen level is controversial. Some authors have reported that a higher initial oxygen content seems to be beneficial to flavor in the early life of the milk due to the reaction with sulfhydryl groups responsible for cooked notes (Nursten, 1997), but after some weeks its effect becomes negligible (Thomas *et al*., 1975). Others have suggested that the presence of oxygen increases the rate of aldehyde formation (Jeon *et al*., 1978) or that it has an unclear influence (Rerkrai *et al*., 1987). Regarding packaging materials, overall flavor of UHT milk protected with aluminum foil (Mehta & Bassette, 1980; Rysstad *et al*., 1998) or packaged in aseptic pouches with oxygen-scavenging film (Perkins *et al*., 2007) and in PET and HDPE bottles with light barrier (Mestdagh *et al*., 2005; Smet *et al*., 2009) was well maintained, and the appearance of staleness was delayed compared with other types of packaging.

Certain markers of the Maillard reaction such as furosine and furfural have been assessed in UHT milk since they are evidence to the use of low-quality raw material, the application of too rigorous conditions of processing, or inadequate storage (Ferrer *et al*., 2000a). Maillard reaction does not give rise to flavor changes in the case of UTH milk treated by conventional processes (van Boekel, 1998).

In reconstituted UHT milk, the quality of milk powder largely determines its sensory attributes. Rates of enzymatic

and oxidative reactions were higher in those milks processed from older powders, and the taste of reconstituted UHT milk was more affected by lipolysis than by proteolysis. Besides, an astringent flavor described as powdery or chalky, and bitter was detected towards the end of storage time whereas lipolytic rancidity could be perceived earlier during storage (Celestino *et al*., 1997b).

15.3.4.2 Milk powder, sterilized, and concentrated milk

The sensory quality of milk powder is greatly affected by the initial milk quality, processing variables, storage conditions, packaging type, presence of physical and chemical agents (oxygen, light, water activity, antioxidants), and the extent of post-processing contamination. Flavor and the volatile profile of dried milks can also undergo seasonal variation and change during storage. A study revealed that WMP manufactured in summer had significantly higher levels of hexanal, pentanal, and dimethyl sulfide than that obtained in autumn and winter. Butanoic acid showed also significant differences between autumn and spring (Biolatto *et al*., 2007).

During storage an overall decrease in milk powder quality is evidence of a direct consequence of off-flavor development. Flavor variability in milk powder may negatively impact consumer acceptability when it is used in reconstituted milk or in ingredient applications (Caudle *et al*., 2005).

Owing to the low water activity that characterizes milk powder, it is accepted that microbial growth does not take place (Chen *et al*., 2003). However, heat-stable enzymes from spoilage bacteria in raw milk retain their activity after thermal processes such as evaporation and spray drying, consequently reducing its shelf-life. Lipolysis can occur in dried products in spite of the low moisture level. Powder manufactured from raw milk kept refrigerated for 48 hours had a higher lipolysis than the product made with fresh raw milk (Celestino *et al*., 1997a). Levels of short-chain fatty acids above the threshold values were also reported in WMP after storage for 2 weeks at 37°C (Chen *et al*., 2003). Astringency and bitterness caused by action of proteases on β-casein (Harwalkar, 1972) or by interaction between whey proteins, calcium phosphate, and caseins (Karagül-Yüceer *et al*., 2002) is a defect that can also be detected in high-heat-treated milk such as sterilized milk, concentrated milk and dry milk products (Lemieux & Simard, 1994; Karagül-Yüceer *et al*., 2002).

Lipid oxidation and non-enzymatic browning are the main sources of off-flavor in dried and sterilized concentrated products (Chen *et al*., 2003; Farkye, 2006), since lipid oxidation is the main factor limiting the shelf-life of WMP. Grassy, painty, staleness, and oxidized are typical off-flavors reported in WMP linked to lipid oxidation. During storage of WMP it was observed that as concentrations of certain compounds such as dimethyl sulfide, branched-chain aldehydes, straight-chain aldehydes, 1-octen-3-ol and 3-octen-2-one increased, grassy and painty flavors increased but other flavors such as cooked, sweet aromatic, and milk fat flavor decreased (Lloyd *et al*., 2009b). Taking into account that milk fat has a key role in the development of flavor defects, the kinetics of the formation of volatile fat oxidation products and other volatile compounds in WMP has been investigated (Hall *et al*., 1985). The heat treatment of milk prior to the manufacture of milk powder is the major factor controlling the oxidative damage. High temperatures of preheat treatments increase the antioxidative capacity but the cooked flavor is intensified (Stapelfeldt *et al*., 1997; Lloyd *et al*., 2009b). Packaging materials must be carefully chosen protecting the powder from moisture, oxygen, light, contamination, and microorganisms (Farkye, 2006). It has been documented that the presence of air in WMP samples packaged in plastic laminate pouches promotes the development of grassy and painty notes whereas nitrogen flushing extends the shelf-life. Therefore, packaging headspace oxygen should be maintained as low as possible to prevent off-flavor (Lloyd *et al*., 2009a). Storage at temperatures above room temperature accelerates autoxidation processes (Stapelfeldt *et al*., 1997); in this sense, refrigerated storage has been proposed to prolong the stability of WMP by delaying oxidative deterioration (Lloyd *et al*., 2009a). The incidence of water activity on oxidative stability seems to be affected by storage temperature and moisture level (Stapelfeldt *et al*., 1997; Farkye, 2006).

Carbonyl compounds and mainly straight-chain aldehydes are considered useful markers to monitor flavor defects of WMP caused by lipid autoxidation (Ulberth & Roubicek, 1995). Hexanal level was reported as the best predictor of grassy flavor, and hexanal or nonanal concentrations were considered good predictors of painty flavor (Lloyd *et al*., 2009b). Some values of sensory thresholds of flavor defects in reconstituted WMP are available in the literature (Hough *et al*., 1992).

The Maillard reaction in dried milk products is much faster than in fluid milk due to the lowest water activity and the storage at room temperature (van Renterghem & De Block, 1996; van Boekel, 1998). Furosine formation was proposed as a marker for this reaction, as its level is highly affected by the drying process and storage conditions (van Renterghem & De Block, 1996). The Maillard reaction has been especially investigated as another source of compounds responsible for staleness in milk powder. Benzothiazole and *o*-aminoacetophenone were reported as the main contributors (Arnold *et al*., 1966; Karagül-Yüceer

et al., 2002). However, other authors have indicated that stale is a composite off-flavor, with at least 12 compounds (including alkylpyrazines, aromatic aldehydes and pyrroles) involved in this defect (Ferretti & Flanagan, 1972).

In addition to lipid oxidation and the Maillard reaction, other chemical reactions can generate the onset of undesirable notes in milk powder. One of the first atypical flavors identified in dry milk was the result of chemical reactions involving ozone in the dryer air; 6-*trans*-nonenal is the major contributor to this defect (Parks *et al*., 1969). More recently, compounds thermally generated such as pyrrolines, thiazolines, and thiazoles have been suggested as possibly responsible for cereal-type off-flavor (Karagül-Yüceer *et al*., 2002). Methional has also been reported as an off-flavor compound in milk powder, whose aroma was described as boiled potato-like. In this product, methional can come either from Strecker degradation or light action on methionine (Karagül-Yüceer *et al*., 2001, 2002). Other compounds such as *p*-cresol and skatole may be contributors of animal-like, cowy, or fecal off-flavors, and their origin seems to be related to cow feeds (Karagül-Yüceer *et al*., 2002). Similarly, an undesirable odor described as cowbarn-like was reported, but its cause could not be elucidated. Tetradecanal, β-ionone, and benzothiazole were the compounds more closely related to this defect (Shiratsuchi *et al*., 1994b). Fortification of non-fat milk powder with vitamin A may often generate a typical hay-like off-flavor, which has been attributed to products formed by thermal oxidation of vitamin A palmitate (Suyama *et al*., 1983).

15.3.4.3 Infant formula

In infant formulae, sterilization or dehydration is absolutely necessary to provide an adequate bacteriological safety and to prolong their shelf-life (Albalá-Hurtado *et al*., 1998; O'Callaghan *et al*., 2011). However, these processes can affect the sensory characteristics. Similarly to milk powder, the main defects are led by Maillard reactions and fat oxidation. Infant formulae combine a number of factors that favor non-enzymatic browning: high quantities of lactose and lysine, relatively high temperatures, and a long time of storage (Ferrer *et al*., 2000b; Chávez-Servín *et al*., 2005). The formation of furfural compounds at advanced stages of Maillard reactions is considered a useful parameter to evaluate the degree of non-enzymatic reactions in infant formulae (Ferrer *et al*., 2000b). Numerous studies have shown a significant increase in furfural levels in infant milks during different conditions of storage (Albalá-Hurtado *et al*., 1998; Ferrer *et al*., 2000b, 2002, 2005; Chávez-Servín *et al*., 2005). On the other hand, the end of shelf-life in powder infant formulae is also affected by fat oxidation. Straight-chain aldehydes originating from unsaturated fatty acid degradation such as propanal, pentanal, and hexanal were proposed as potential indicators of infant milk powder oxidation (Romeu-Nadal *et al*., 2004). Hexanal content was found to vary from roughly 500 to 3500 μg/kg for non-oxidized and oxidized infant formulae, respectively (Fenaille *et al*., 2003).

15.3.5 Volatile profile and sensory characteristics of non-thermally treated milk

Although thermal treatments are considered the more effective methods to destroy microorganisms, some disadvantages related to chemical modifications of milk constituents or changes in the flavor have been claimed. Non-thermal processing technologies such as microfiltration, ultrasonication, pulsed electric field, high pressure, and microwave have emerged as new alternatives that allow inactivation of spoilage and pathogenic microorganisms while maintaining chemical properties of milk. Consumer perception about non-thermal treatments is that they provide more natural or fresher foods than heat treatments. However, at this time, the physical processes employed to reduce microbial loads in milk are unable to reduce the spore counts sufficiently to produce a safe product. As a consequence, they are often applied in combination with heat treatments (Deeth & Datta, 2011a). So far, few studies have been conducted to assess the effect of non-thermal processes on the volatile profile and/or sensory quality of milk. The assayed technologies have been microfiltration (Elwell & Barbano, 2006; Rysstad & Kolstad, 2006), ultrasound (Riener *et al*., 2009; Chouliara *et al*., 2010; Engin & Karagül-Yüceer, 2012), pulsed electric fields (Zhang *et al*., 2011), microwave (Valero *et al*., 1999, 2000; Clare *et al*., 2005), high hydrostatic pressure processing (Vazquez-Landaverde *et al*., 2006b), and ultra-high-pressure homogenization (UHPH) (Pereda *et al*., 2008a).

15.3.5.1 Microfiltration

The development of membrane technology has revolutionized the field of dairy processing (Pouliot, 2008). Microorganisms are removed according to their bacterial size, unlike pasteurization which is designed to destroy any microbiological danger in the food (Rysstad & Kolstad, 2006). Microfiltration has proved its capacity to eliminate bacterial cells and spores as well as to increase shelf-life when it is combined with mild pasteurization (Avalli *et al*., 2004; Deeth & Datta, 2011a). Pretreatment by cross-flow microfiltration of milk with a cell load reduction up to 4 log cfu/mL is used for the production of low heated fluid milks having a flavor similar to that of raw milk and a shelf-life three to five times longer than that of classical products (Saboya & Maubois, 2000; Elwell & Barbano, 2006). However, since the enzymes are

not inactivated by microfiltration, changes in flavor by proteolysis and lipolysis during storage could be expected. This is dependent on initial somatic cell count of raw milk and storage temperature (Elwell & Barbano, 2006).

15.3.5.2 Ultrasound

Ultrasonic treatment has attracted considerable interest in food science and technology. This emerging technology is based on the use of sound waves above the frequency of human hearing (>18 kHz). Over the last decade, an increase in potential applications of ultrasound in the field of food processing and preservation has been observed (Knorr *et al*., 2004; Dolatowski *et al*., 2007). The effects of ultrasonic waves on physicochemical characteristics, sensory properties, shelf-life, enzymes, and microorganisms of milk as well as application in the dairy industry for the homogenization process have been reported (Bermúdez-Aguirre *et al*., 2009; Cameron *et al*., 2009; Ashokkumar *et al*., 2010; Chouliara *et al*., 2010; Engin & Karagül-Yüceer, 2012). Although ultrasonicated milks seem to offer an alternative to pasteurization, many aspects of ultrasound mechanisms such as cavitation phenomena (formation and violent implosion of bubbles) and heating (conversion of acoustic energy to heat) (Villamiel & de Jong, 2000; Deeth & Datta, 2011b) have not been fully elucidated. In addition, milk flavor can be affected by certain chemical reactions that may occur during the sonication process such as oxidation by formation of hydroxyl radicals and hydrogen atoms or pyrolysis (Makino *et al*., 1983). In particular, the generation of volatile compounds in milk treated by ultrasound (Riener *et al*., 2009; Chouliara *et al*., 2010; Engin & Karagül-Yüceer, 2012) has been investigated. Some compounds derived from lipid oxidation such as aldehydes and hydrocarbons appear to be related to this treatment. An undesirable rubbery aroma has been detected in sonicated milks but the responsible components were not yet identified (Riener *et al*., 2009).

15.3.5.3 Pulsed electric field

Pulsed-energy or pulsed electric field has gained increasing attention as an alternative to traditional food processing. This technology utilizes very short electric pulses (nanoseconds to milliseconds) at high electric field intensities at moderate temperatures (Bendicho *et al*., 2002). Electric field strength and treatment time are two of the most important factors involved in pulsed electric field processing (Jeyamkondan *et al*., 1999; Bendicho *et al*., 2002). In the last decade, the effectiveness of different processing parameters on microbial and enzyme inactivation and functional properties of milk has been evaluated (Bendicho *et al*., 2002; Floury *et al*., 2006; Noci *et al*., 2009; Bermúdez-Aguirre *et al*., 2011). In spite of some controversial results, pulsed electric field is considered a promising technology to replace, at least partially, the traditional thermal treatments of liquid foods or to extend the shelf-life of pasteurized milk (Sepulveda *et al*., 2005; Deeth & Datta, 2011b). Few studies have considered the effect of pulsed electric field treatment on volatile fraction. Sampedro *et al*. (2009) observed that pulsed electric field treatment applied to orange juice and milk-based beverages achieved the same degree of microbial and enzyme inactivation as thermal treatment, but better preserved color and volatile profile. Zhang *et al*. (2011) found some differences in the volatile fraction of milks subject to pulsed electric field compared with pasteurization, but these differences were not detected by olfactometric analysis. Little or no change in the sensory properties of milk was also reported (Bendicho *et al*., 2002; Sepulveda *et al*., 2005; Deeth & Datta, 2011b). Therefore, it has been claimed that pulsed electric field processing maintains the freshness of foods (Bendicho *et al*., 2002).

15.3.5.4 Microwave

Microwave treatment has been suggested as a reliable method of heating since the milk is not exposed to overheated exchange surfaces (Clare *et al*., 2005). Results obtained from the comparison of volatile compounds between microwave-heated milks and conventionally heated milks have demonstrated that microwave technology is a useful alternative for milk processing since the sensory characteristics of this milk are equivalent to, if not better than, those exhibited by conventional processing (Valero *et al*., 1999, 2000; Clare *et al*., 2005).

15.3.5.5 High hydrostatic pressure

High hydrostatic pressure or high-pressure processing is being looked at with particular interest because the nutritional and sensory properties of food seem to be not affected (Mussa & Ramaswamy, 1997). Although it has been recognized as an alternative to thermal processing, the resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration and type of microorganisms (Trujillo *et al*., 2002; Fonberg-Broczek *et al*., 2005). The application is generally performed at pressures ranging from 300 to 600 MPa for 2–30 min (Deeth & Datta, 2011c) and at ambient temperature. Like other technologies, the effect of high pressure on microorganism destruction, and physicochemical and sensory properties of milk have been investigated (Pandey *et al*., 2003; Fonberg-Broczek *et al*., 2005; Altuner *et al*., 2006; Huppertz *et al*., 2006). An interesting review about applications of high hydrostatic pressure on milk and dairy products by Trujillo *et al*. (2002) is available. Information about the impact of high

hydrostatic pressure on the volatile fraction of milk and the mechanisms of volatile generation is still very scarce. A small effect on covalent bonds, smaller organic molecules such as volatile compounds and vitamins was reported (Trujillo *et al*., 2002). However, other studies have indicated a strong effect of high hydrostatic pressure on compounds produced from the Maillard reaction (Frank *et al*., 2002). In fact, the effect of high hydrostatic pressure on the volatile profile seems to be highly dependent on the processing temperature. Vazquez-Landaverde *et al*. (2006b) observed that high-pressure processing in the range 480–620 MPa at low temperature (25°C) has a minor effect on volatile components of milk, and particularly methyl ketones and aldehydes were not formed at any applied pressure, suggesting clear advantages compared with thermal treatments. However, a combination of high pressure and high temperature (60–80°C) increased dramatically the concentration of aldehydes. It has been hypothesized that oxygen is more soluble under high pressure and hence it could promote hydroperoxide formation and consequently increase aldehyde production.

15.3.5.6 Ultra-high-pressure homogenization

The most recent development is a new high-pressure process called ultra-high-pressure homogenization (UHPH), which is based on the same principle as conventional homogenization but works at higher pressures (up to 400 MPa) (Pereda *et al*., 2009). Results from microbial inactivation, physicochemical parameters and shelf-life from milk subject to UHPH processes indicate its suitability to replace the conventional thermal treatments (Pereda *et al*., 2007, 2008b, 2009; Pedras *et al*., 2012). Comparison of volatile profiles between milk samples subjected to thermal processing (pasteurization, UHT and sterilization) and different UHPH conditions revealed that whereas heat treatments produced an increase in aldehyde and methyl ketone contents as thermal intensity increased, UHPH technology induced an increase in aldehydes alone, which was more pronounced as the value of the applied pressure increased (Pereda *et al*., 2008a).

15.4 MILK FROM MONOGASTRIC SPECIES

Monogastric species, such as humans and horses, produce milks with nutritional, sensory, and physicochemical characteristics that differ from the milk of ruminant species, while the camel is considered a pseudo-ruminant with a stomach anatomy similar to, but modified from, that of true ruminants. Human and camel milks have been the most studied, because of their relative importance compared to other species. An example of differences in composition to bovine milk is the fact that β-casein is the major protein in camel and human milk, and they do not contain β-lactoglobulin (El-Agamy, 2006; Morgan, 2006; Konuspayeva *et al*., 2009; Al-haj & Al-Kanhal, 2010).

Sensory properties of camel milk include an opaque and white color and acceptable taste, which is normally described as sweet, sharp, and sometimes salty. The taste changes according to the type of plant consumed by the camels and availability of water (Al-haj & Al-Kanhal, 2010). In turn, sensory evaluation of fresh human milk has revealed very low odor intensity (practically odorless), and its attributes were described as predominantly sweet, fatty and soybean-like, with slight buttery and cooked milk-like notes (Spitzer & Buettner, 2010).

The volatile profile of human milk is the result of the delicate balance of several compounds of different chemical classes such as aldehydes, ketones, fatty acids, and terpenes (Sandgruber *et al*., 2011). Analysis of human milk samples carried out by Shimoda *et al* (2000) has shown a volatile profile quite different from that of cows' milk. Aldehydes were the most important group of compounds in human milk among which unsaturated and long-chain carbonyl compounds were the most representative of the volatile fraction. In addition to these compounds, lactones and fatty acids are commonly found (Spitzer & Buettner, 2010). The main difference in flavor perception between human and bovine milk seems to be sweetness, which is higher in human milk (McDaniel *et al*., 1989). When samples of both types of milks are stored at –19°C, significant differences in the intensities of other attributes such as hay and fishy flavor have been observed (Spitzer & Buettner, 2010).

Today, breastfeeding is often performed by pumping the mother's milk and storing it until use, which can result in flavor changes or in development of harming constituents. Human milk seems to be a food matrix highly sensitive to sensory deterioration, being mainly affected by oxidative and lipolytic processes that involve free fatty acids (Sandgruber *et al*., 2012). In this regard, the analysis of several storage conditions on the stability of the triacylglyceride fraction of human milk indicated that storage only at –20°C without previous heat treatments led to the appearance of free fatty acids (Morera Ponds *et al*., 1998). Recently, several studies have been conducted on the influence of different storage conditions on human milk flavor. Spitzer and Buettner (2010) evaluated the sensory changes in human milk samples after 2 months' storage at –19°C. They found that the aroma profile of breast milk can be considerably affected by storage at the recommended conditions. Several volatile compounds such as 1-octen-3-one, (Z)-1,5-octadien-3-one, *t*-4,5-epoxi-(E)-z2-decenal, (E,Z,Z)-2,4,7-tridecatrienal, (E,Z)-2,4-nonadienal, and (E,Z)-2,4-decadienal were significantly increased in stored samples. Some of these carbonyl compounds could be responsible for the intense and characteristic fish-like and

metallic off-flavors that develop in this type of milk. In addition, rancid, soapy, and sweaty notes were detected, which were attributed to increased concentration of free fatty acids. In a subsequent work, Spitzer *et al*. (2010) reported that the heating of milk prior to freeze storage induced slight flavor changes, with generation of an egg-white-like note but with no further generation of off-flavor during storage. Sandgruber *et al*. (2012) observed a delay in off-flavor development by oxidative processes if storage of human milk is performed at –80°C.

Taking into account the important variations observed in specific odorants between milks of different mothers, maternal diet has been proposed as another possible source of flavor changes in milk, mainly due to transmitted compounds. A recent study investigated whether specific fish oil odor constituents can be transferred to mothers' milk (Sandgruber *et al*., 2011). Surprisingly, data obtained from sensory profiles and chemical markers showed that no statistical differences occurred between milks from mothers after long-term fish oil supplementation and the control group. Today, this topic is being considered.

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16
Fermented Milk and Yogurt

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16.1 GENERAL ASPECTS OF FERMENTED MILK

The great variety and diversity of current fermented dairy products seems to be a reflection of human history from ancient times to the present. Yogurt, the most widely fermented milk product worldwide, is similar to several traditional products that were manufactured thousands of years ago. However, where possible, fermentation and storage conditions should follow strict hygienic and formulation guidelines to ensure good quality and safety of old and new dairy products.

The information collected in this chapter covers general aspects of fermented milk, from traditional foods to current products, and also standards and regulations of fermented milk, and health benefits.

16.1.1 Yogurts

Yogurt is a major dairy product made from the fermentation of milk. The bacteria used to make yogurt are known as "yogurt starter cultures." These cultures ferment lactose and produce lactic acid, which acts on milk proteins to give yogurt its texture and characteristic properties. No one knows where or how yogurt originated, but around 10 000 BC the human way of life changed from food gathering to food producing and this change included the domestication of cows, sheep, goats, buffaloes, camels, yaks, horses, and even reindeer. Although the primary function of fermentation is to extend the shelf-life of milk products, this process also improves the taste of milk and enhances the digestibility of the product (Kosikowski & Mistry, 1997).

16.1.1.1 Types of yogurt

Yogurt products can be classified into about five categories:

1. Set type yogurt is incubated and cooled in the final package and is characterized by a firm jelly-like texture.
2. Stirred type yogurt is incubated in a tank and the final coagulum is broken by stirring prior to cooling and packing. The texture of stirred yogurt will be less firm than a set yogurt, somewhat like a very thick cream.
3. Drinking type yogurt is very similar to stirred yogurt, having the coagulum broken prior to cooling. In a drinking yogurt the agitation used to break the coagulum is severe. Little if any reformation of the coagulum will occur after packing.
4. Frozen type yogurt is inoculated and incubated in the same manner as a stirred yogurt. However, cooling is achieved by pumping through a whipper/chiller/freezer in a fashion similar to ice cream. The texture of the finished product is mainly influenced by the whipper/freezer and the size and distribution of the ice crystals produced.
5. Concentrated yogurt is inoculated and fermented in the same manner as a stirred yogurt. Following the breaking of the coagulum, the yogurt is concentrated by boiling off some of the water; this is often done under vacuum to reduce the temperature required. Heating of low pH yogurt can often lead to protein being totally denatured and producing rough and gritty textures. This is often called "strained" yogurt because the liquid that is released from the coagulum upon heating used to be strained off in a manner similar to making soft cheese.

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Table 16.1. Output development in selected countries (liquid milk and fermented products).

	2009 (million tonnes)	Annual growth 2008/2009 (%)
Liquid milk		
EU 27	32.8	0.2
USA	25.2	0.6
China	13.2	4.6
Brazil	10.9	2.0
India*	7.9	4.8
Mexico	4.5	3.3
Russia	4.3	1.0
Japan	3.9	–3.7
Fermented products and milk drinks		
EU 27	10.4	0.4
China	3.2	22.5
USA	2.5	4.7
Japan	2.3	–1.6
Russia	2.2	0.0
Turkey†	2.1	0.7
Iran	0.9	4.0
Argentina	0.6	1.5

* Figures only for cooperatives.

† 2008 figures.

Source: reproduced from International Dairy Federation (2010), with permission of IDF Press.

16.1.1.2 Production and consumption

Currently, the most popular yogurts are all-natural, premium, Greek-style, functional, and organic products. The increased production of fermented products is currently more consistent than the production of liquid milk in most countries (Table 16.1). This growth was especially impressive in the USA (+4.7%) and in China (+22.5%).

An explosion in the functional, natural, premium, and organic products has helped generate interest in yogurt and raised its retail prices. Despite several years of rising consumption, yogurt drink sales fell in 2009 due to premium pricing. The perception that yogurt drinks are high in calories has also led consumers to switch to lighter versions or consume yogurt drink products less often or not at all.

Refrigerated yogurt consumption among adults increased from 52% in 2003 to 56% in 2009, especially among those aged 25–44 years in the USA. A greater proportion of women (68%) eat refrigerated yogurt or yogurt drinks compared with men (43%). Teenagers consume the most refrigerated yogurt products among any other group (59%). Yogurt usage among ages 6–11 years reached 54% in 2009, which was driven by an influx of products targeted at a younger demographic. The two most important attributes consumers consider when purchasing yogurt are flavor selection and price (International Dairy Federation, 2010).

Consumers are most concerned with the calorie content of yogurt products, which was cited by 50% of respondents. High calcium, live active cultures, low sugar, and-all natural also ranked among the most important attributes of yogurts by consumers.

Yogurt consumption is on the rise, driven by healthy-eating trends and a growing awareness of the health benefits of yogurt. Low-fat yogurt is the most popular, followed by regular yogurt, which are consumed by 54% and 47% of yogurt eaters, respectively. Frozen yogurt consumption had declined since 2003, but sales have recently grown.

16.1.1.3 Recent new product trends

The recent trends in the yogurt market include consumption of organic and natural, functional yogurts made with probiotics, high protein and fiber, Greek-style yogurt, dessert-style yogurts, and child-targeted products. Whole grain, a major trend in other categories, has recently had success in the yogurt market.

About 1200 new yogurt products have been introduced in the USA since 2005. The refrigerated yogurt category accounts for two-thirds of new products. Although product introductions decreased in 2009, refrigerated yogurts still led the number of rollouts. Frozen yogurt was the only segment to show an increase in new products since 2008. A low-fat yogurt, a kosher yogurt, and vitamin- and mineral-fortified yogurt were the top three new yogurt products in 2009. Since 2008, yogurts containing no additives or no preservatives, and high-protein yogurts have been the only three products to show increases, which points to some emerging trends (International Dairy Federation, 2010). Despite yogurt being the main probiotic on the market, several other fermented dairy products have become well established worldwide (Table 16.2).

16.1.1.3.1 Greek style yogurt

Greek-style yogurt is yogurt which has been strained in a cloth or paper bag to remove the whey, giving a consistency between that of yogurt and cheese, while preserving yogurt's distinctive sour taste. It is relatively high in milk fat and milk solids-not-fat. It has a creamy texture and mild flavor as a result of whey removal by centrifugal/membrane separation or by straining through cloth (Chandan & O'Rell, 2006).

The base for this type of yogurt is whole milk, supplemented with cream to standardize the fat level to 7%. In traditional processing, after fermentation is complete the yogurt is concentrated by straining through cheesecloth at 4°C overnight. Because of the drainage of whey, the total

Table 16.2. Worldwide fermented dairy products.

Name	Type of milk	Microorganism	Country	Characteristics
Yogurt	Buffalo, cow, goat, sheep	*Lactobacillus bulgaricus* *Streptococcus thermophilus*	Common	Plain, sweetened, carbonated, and flavored milk
Cultured buttermilk	Buffalo, cow, goat, sheep	*Streptococcus lactis* *Streptococcus cremoris*	Common	Valued as a recipe ingredient and digestive aid Pleasantly tart and smooth taste
Curd	Buffalo, cow	*Lactococcus lactis* *Lactobacillus delbrueckii* *Lactobacillus plantarum*	Common	High acidity and fat contents, sour fermented milk
Acidophilus milk	Cow, goat	*Lactobacillus acidophilus*	Common	Fermented milk using *Lactobacillus acidophilus* Tastes tangy and thicker than regular milk
Lassi	Buffalo, cow	*Lactobacillus bulgaricus*	India	Made by blending yogurt with water and Indian spices Used as a folk remedy for gastroenteritis
Kefir	Cow, goat, sheep	*Lactobacillus kefir* *Streptococcus lactis* *Leuconostoc* spp. Yeast	Central Asia and Europe	Alcohol containing fermented milk (kefir grain) Low molecular weight peptide is higher than other fermented milk
Koumiss	Mare milk	*Lactobacillus acidophilus* *Lactobacillus bulgaricus* Yeasts	Central Asia	Milky gray color, sharp alcoholic and acidic taste Liquid starter culture is used
Leben	Goat, sheep	*Streptococcus lactis* *Streptococcus thermophilus* *Lactobacillus bulgaricus*	Middle East North Africa	Live cultures are absent Tarter, thinner, and less smooth than yogurt

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solids increase from 14% to 21–23% (Robinson, 2002). In Greece, strained yogurt is traditionally made from sheep milk. More recently, cow milk has often been used, especially in industrial production.

16.1.2 Other fermented bovine milk products

16.1.2.1 Cultured buttermilk

This product was originally produced after churning butter from sweet or sour cream made from the fermentation of the butter milk, but today it is commonly produced from skim or whole milk. Cultured buttermilk is a fermented milk product made by adding a starter culture to milk. During this process, starter cultures are inoculated into cow milk, which results in an increase in acidity due to lactic acid formation during the fermentation of the lactose sugar in milk. In addition, this fermentation process causes the casein in milk to precipitate and curdle. Through this process, the cultured buttermilk becomes sour and thicker than plain milk. Also, the acidity increases the refrigerator shelf-life because it inhibits the growth of pathogenic bacteria.

During the processing of cultured buttermilk, the supplied milk must be fresh (White, 1977, 1979) and cannot contain inhibitor substances such as antibiotics and sanitizers. The key step in this process is the addition and development of a starter culture to increase the acidity and flavor. *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* has been used as the starter culture. After fermentation, the resulting curd is broken and stirred slowly, cooled, and slightly salted. Fruit condiments, essences, and butter flakes can be added to plain cultured buttermilk.

Cultured buttermilk is largely a consumer product in Europe, but in the USA very large quantities are produced and used as ingredients. For instance, it provides an excellent base for making various kinds of dressings and baked goods, which require a smooth tangy flavor. In addition, it can be packaged for sale in the retail trade.

16.1.2.2 Cultured cream

Cultured cream is produced from the manufacturing of cultured buttermilk. It has a clean lactic flavor and fresh aroma. The fat content of cultured cream is standardized from 12% to 30% depending on the required properties (Parker, 2003). The starter is similar to that used for cultured buttermilk. The cream after standardization is usually pasteurized at 75–80°C and homogenized at above 13 MPa to improve the texture. Cultured cream is inoculated with 2–5% lactococci (*Lactococcus lactis* subsp. *lactis* and *cremoris*) and incubated for 16–18 hours at 22°C until the acidity approaches 0.6%.

Cultured cream with 18% fat and 9% milk solids is consumed in North America, while in France and other European countries products having a fat content of 35–50% are more popular (Parker, 2003). Cultured cream is manufactured by large food companies and used in a variety of recipes, as a dressing on salads, a topping on fruit and cheesecake, and as a stabilizer.

The latest research on cultured cream involves the manipulation of *Lactococcus lactis* strains by culturing (Garcia *et al*., 1998) and genetic engineering techniques (Hugenholtz *et al*., 2000) to overproduce diacetyl as a flavor. Cultured cream products will continue to grow as these production challenges are met.

16.1.2.3 Acidophilus milk

Acidophilus milk is a traditional milk fermented with *Lactobacillus acidophilus*, which has therapeutic benefits in the gastrointestinal tract. Skim or whole milk is heated to a high temperature, for example 95°C for 1 hour, to reduce the microbial load and flavor. Milk is inoculated at a level of 2–5% with starter culture and incubated at 37°C until coagulated, but some acidophilus milk is not coagulated. Some acidophilus milk has an acidity level as high as 1% lactic acid, but for therapeutic purposes 0.6–0.7% is more common. Another variation has been the introduction of sweet acidophilus milk, where the starter culture has been added without an incubation period. It is thought that the culture will reach the gastointestinal tract, where its therapeutic effects will be realized, but the milk has no fermented qualities; thus, the lack of high acidity and flavor is considered undesirable by some people (Vedamuthu, 2006).

16.1.2.4 Kefir

Kefir is popular in Russia, some Balkan countries, and the Near and Middle East, and originated in the area abutting the Caucasus mountain range. Traditional kefir is produced using kefir grains as inoculum. Kefir is an acidic alcoholic product since both lactic acid bacteria and yeasts are involved in the fermentation. Kefir may be made from goat, sheep, or cow milk (Kosikowski & Mistry, 1997). The lactic acid content is usually around 0.8% and the alcohol level is about 1.0%. Carbon dioxide is the other major fermentation by-product in kefir.

Kefir is made from whole milk heat treated at 95°C for 5 min. This process denatures whey proteins and improves product consistency. Homogenization is also used in modern practice. A portion of the processed milk is used to prepare the inoculum, which is mixed with kefir grains and incubated at 20–25°C for 20 hours. At the end of this period the kefir grains are removed by sieving and rinsed in cold water for re-use. The cultured milk is then used as a starter at a level of 3–5% and fermentation is carried out at 20–25°C for 20 hours. The composition of the starter culture varies, and can include *Lactococcus lactis* subsp. *lactis* and subsp. *cremoris*, *Lactobacillus acidophilus*, *Lactobacillus kefir*, *Lactobacillus casei*, *Candida kefyr*. The kefir is then held for several hours during which time the coagulum stabilizes and the final product contains 0.9–1.1% lactic acid and 0.5–1.0% ethanol.

Kefir has had a long history of health benefits in Eastern European and Middle Eastern countries, where it is associated with general well-being. Kefir can reduce symptoms of lactose intolerance by providing an extra source of β-galactosidase. Although the mechanism of action is not yet known, kefir has been shown to inhibit and suppress several types of cancer and metastasis. These effects are thought to be mediated through the exopolysaccharides and antioxidative properties (Shiomi *et al*., 1982; Cross *et al*., 2002). The beneficial action of kefir may also be mediated through different bioactive peptides formed during fermentation or digestion.

16.1.2.5 Other fermented milk products

There are many other fermented dairy products, including vilii, dahi, skyr, and beverages, based on *Lactobacillus bulgaricus* or *Bifidobacterium* strains and a host of others (Vedamuthu, 2006). These represent the great diversity of cultured dairy products produced around the world. This diversity not only reflects the geographical region of origin but also the types of milk used in their production, the gradation in the technology employed, the cultural conditions, and the types and species of microbiota involved in these fermentations.

16.1.3 Fermented milk and yogurt products from other dairy species

16.1.3.1 Fermented goat milk products

Goat milk is generally not used to make industrially prepared fermented milk products, but individual goat farms market yogurt and fermented milk products such as kefir. Goat milk is perfectly suited for the production of fruit and

cereal yogurt known commercially under various names, such as Bircher Muesli, fruit, sports, and all-fruit yogurt.

The basic composition of goat milk is similar to that of cow milk and on average contains 12.2% total solids, consisting of 3.8% fat, 3.5% protein, 4.1% lactose, and 0.8% ash. Goat milk has slightly less total casein but more non-protein nitrogen than cow milk (Park, 2006a). Goat milk differs from cow or human milk in higher digestibility, distinct alkalinity, higher buffering capacity, and certain therapeutic values in human medicine and nutrition (Haenlein & Caccese, 1984; Park, 1990, 1992, 1994).

Goat milk fermented products are prepared from goat milk only (usually in liquid form) such as airan, goat milk liquid, firm yogurt, and those prepared from mixtures of goat, sheep and/or cow milk. Nowadays, goat milk dairy products have been rediscovered as fitting well the new interest in healthy foods. Goat milk has been used successfully in cases of cow milk allergies and by patients with various metabolic and gastrointestinal ailments. Goat milk proteins can differ genetically from some cow milk proteins, and goat milk fat usually has a better profile of fatty acids. Goat milk cheeses have acquired a worldwide gourmet reputation, and demand is growing (Park & Haenlein, 2006).

16.1.3.2 Fermented sheep milk products

Sheep milk has higher specific gravity, viscosity, refractive index, and titratable acidity, and lower freezing point than average cow milk (Haenlein & Wendorff, 2006). Lipids in sheep milk have higher physical characteristics than those in cow milk, but there are variations among the different reports (Anifantakis, 1986; Park, 2006a). Sheep milk yogurt is made from whole filtered milk, which is pasteurized at 95°C, then filled into containers, and cooled and inoculated with a yogurt culture. Sheep milk has higher total solids and major nutrient contents than goat and cow milk. Sheep colostrum in the early postpartum period is also higher in basic nutrients than cow colostrum: fat 13.0% vs. 5.1%, protein 11.8% vs. 7.1%, lactose 3.3% vs. 3.6%, minerals 0.9% vs. 0.9%, and total solids 28.9% vs. 15.6%, respectively (Anifantakis, 1986). Changes in sheep milk compositions occur by season, because towards the end of lactation, the fat, protein, solids and mineral contents increase, while the lactose content decreases (Brozos *et al*., 1998; Haenlein, 2001, 2004).

16.1.3.3 Fermented buffalo milk products

In general, buffalo milk contains higher proportions of all major constituents than cow milk. The majority of compositional studies are on the milk from Murrah buffaloes, and this contains 16.7% total solids, consisting of 7.0% fat, 4.0% protein, 5.1% lactose, and 0.8% ash. Because of its higher fat, solids-not-fat, and total solids contents, it yields relatively more cream, butter, cheese, condensed milk, and other dairy products (Pandya & Khan, 2006).

The ratio of fat, protein, lactose and ash in buffalo milk is different from that in cow milk. This can affect processing and may need to be standardized to maximize yield. The higher proportion of solid fat makes buffalo butter harder and less spreadable. The hydrolysis of fat is slower in buffalo milk, which affects ripening of cheeses. Buffalo milk casein micelles are larger and contain less κ-casein, which makes the primary phase of rennet action slower, while the secondary phase is faster because of more calcium. Buffalo milk curd loses moisture quickly; as a result, the cheeses are hard and dry. Slower proteolysis leads to more time in development of cheese flavor, body, and texture (Sahai, 1996).

16.1.3.4 Fermented mare milk products

Horses have been traditionally used as dairy animals in central Asia, Mongolia, and the former Soviet Union, where mare milk has been one of the important food sources for the human populations in these regions. The milk has mainly been used for the manufacture of a lactic alcoholic beverage, named Koumiss, and cheese (Orskow, 1995; Montanari *et al*., 1996; Marconi & Panfili, 1998). It is estimated that 30 million people throughout the world drink horse milk more or less regularly (Dereau & Martin-Rosset, 2002). The protein in mare milk is different from the protein in the milk from other species: mare milk contains less casein and fat than cow milk, and does not coagulate (Vedamuthu, 2006). Lactic acid bacteria and yeasts are both responsible for acidification and the final sensorial properties of the koumiss product. *Lactobacillus casei*, *Lactobacillus helveticus*, and *Lactobacillus plantarum* have been used as starter cultures. The final product contains about 0.7–1.8% lactic acid and 1.0–2.5% ethanol and is not curdled (Vedamuthu, 2006). In modern manufacturing, cow milk is often used instead of mare milk as the starting material. In traditional manufacturing, koumiss was used not only as a nutritive food, but also as an ancient medical remedy. Research on the health benefits of koumiss has yet to be conducted, but the microbial similarity of this product with kefir indicates that it may contain similar bioactive compounds of microbial origin (exopolysaccharides, peptides) as kefir.

Currently, koumiss is manufactured at an industrial level in these countries (Tamime *et al*., 1999). The composition of mare milk is significantly different from that of cow milk (Marconi & Panfili, 1998; Malacarne *et al*., 2002; Kucukcetin *et al*., 2003). Mare milk is similar to human milk, with particular reference to its low nitrogen content, its low ratio of casein to whey

protein, and its high content of lactose (Bonomi *et al*., 1994). Furthermore, certain characteristics including high polyunsaturated fatty acids and low cholesterol content appear to support the interest in increasingly using mare milk for human consumption (Iametti *et al*., 2001).

16.2 STANDARDS AND REGULATIONS

Each country has its own standards with regard to the regulation of fermented milk. Some of these standards are similar, but others are different, such as milk protein content and milk fat level, grouping milk fat and milk solids-not-fat. The regulations and standards for fermented milk in various countries are listed in Table 16.3.

16.2.1 International Codex Standard

16.2.1.1 Description

Fermented milk is a product obtained by the fermentation of milk, which may have been manufactured from milk with or without compositional modification, by the action of suitable microorganisms, and resulting reduction in pH with or without coagulation (isoelectric precipitation). These starter microorganisms must be viable, active, and abundant in the product at the date of minimum durability. If the product is heat treated after fermentation, the requirement for viable microorganisms does not apply (Codex Standard 243-2003).

Raw materials allowed in the Codex Standard of Fermented Milk are limited to milk and milk products obtained from milk and potable water used for reconstitution. Additional permitted ingredients include starter cultures and sodium chloride. Gelatin and starch are only allowed in heat-treated fermented milk, flavored fermented milk, and plain fermented milk.

16.2.1.2 Composition

Fermented milk is divided into four groups: fermented milk, yogurt and acidophilus milk, kefir, koumiss. Codex regulations require standards for level of protein, fat, acidity, and microorganisms. Specific composition requirements for various Codex fermented milk and allowable food additives in fermented milk are listed in Tables 16.4 and 16.5.

16.2.2 USA, Australia and New Zealand, and Europe

16.2.2.1 Description

Yogurt is produced by culturing one or more of the optional dairy ingredients (cream, milk, partially skimmed milk, or skim milk) with a bacterial culture that contains lactic acid-producing bacteria, i.e., *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. One or more of the other optional ingredients may be used (US FDA-21 CFR part 131-203).

Fermented milk means a milk product obtained through the fermentation of milk and/or products derived from milk, where the fermentation involves the action of microorganisms and results in coagulation and a reduction in pH. According to Australia & New Zealand Food Standard 2.5.3, yogurt means fermented milk where the fermentation has been carried out with lactic acid bacteria (Table 16.6).

Table 16.3. Regulations for yogurt in various countries.

Country	Milk protein (%)	Milk fat (%)	Milk solids-not-fat (%)	Titratable acidity (%)	Starter culture microorganism (cfu/g)
Codex*	≥2.7	≤10		≥0.3	$\geq 10^7$
USA†	≥3.25	≥8.25	≥0.9		
Australia‡	≥3				$\geq 10^6$
Europe§	≥2.7	≤10		≥0.3	$\geq 10^7$
China¶	≥2.9	≥3.1	≥8.1		
Japan**			≥8.0		$\geq 10^8$
Korea††			≥8.0		$\geq 10^8$

*Codex Standard for Fermented Milk (243-2003).
†US FDA Code of Federal Regulations part 131–203.
‡Australia & New Zealand Food Standard 2.5.3.
§European Union Council Regulation No. 178/2002.
¶China National Dairy Standard GB2746-1999.
**Ministry of Health and Welfare Ordinance No. 52, Japan.
††Livestock Product Processing Control Act 2.1.4, Korea.

Table 16.4. Composition of fermented milk for Codex requirement (Codex Standard 243-2003).

	Fermented milk	Yogurt, alternate culture yogurt and acidophilus milk	Kefir	Kumiss
Milk protein* (% m/m)	min. 2.7%	min. 2.7%	min. 2.7%	
Milk fat (% m/m)	<10%	<15%	<10%	<10%
Titratable acidity, expressed as % lactic acid (% m/m)	min. 0.3%	min. 0.6%	min. 0.6%	min. 0.7%
Ethanol (% vol./w)				min. 0.5%
Sum of microorganisms constituting starter culture (cfu/g, in total)	min. 10^7	min. 10^7	min. 10^7	min. 10^7
Labeled microorganisms† (cfu/g, total)	min. 10^6	min. 10^6		
Yeasts (cfu/g)			min. 10^4	min. 10^4

*Protein content is 6.38 multiplied by the total Kjeldahl nitrogen determined.

†Applies where a content claim is made in the labeling that refers to the presence of a specific microorganism.

Source: reproduced from FAO, with permission (www.codexalimentarius.org/input/download/standards/400/CXS_243e.pdf. Accessed 9 January 2013).

Table 16.5. Allowable food additives for Codex Standard of fermented milk (Codex Standard 243-2003).

Additive class	Fermented milk		Fermented milk heat-treated after fermentation	
Colors	**Plain**	**Flavored**	**Plain**	**Flavored**
Sweeteners	—	O	—	O
Emulsifiers	—	O	—	O
Flavor enhancers	—	O	—	O
Acids	—	O	O	O
Acidity regulators	—	O	O	O
Stabilizers	X	O	O	O
Thickeners	X	O	O	O
Preservatives	—	—	—	O
Packaging gases	—	O	O	O

O, justified; —, not justified; X, restricted to reconstitution.

Source: reproduced from FAO, with permission (www.codexalimentarius.org/input/download/standards/400/CXS_243e.pdf. Accessed 9 January 2013).

Table 16.6. Australia and New Zealand standards for fermented milk.

Component or parameter	Proportion
Protein (measured as crude protein)	Minimum 30 g/kg
pH	Maximum 4.5
Microorganisms from the added culture	Minimum 1 000 000 cfu/g

Source: reproduced from the Commonwealth of Australia (http://www.comlaw.gov.au/Details/F2011C00622. Accessed 9 January 2013).

In Europe, fermented milk is a milk product obtained by the fermentation of milk, such as yogurt, by the action of suitable microorganisms and resulting reduction in pH with or without coagulation (European Union Council Regulation No. 178/2002).

16.2.2.2 Composition

A provision requiring ingredients may be added, where one or more of the ingredients specified in the paragraph must have a minimum fat level of 3.25% before the addition of bulky flavorings. Also there is a minimum requirement of 8.25% milk solids-not-fat, besides 2000 IU vitamin A (optional) and 400 IU vitamin D (optional) per quart (0.946 L). The fermented milk products do not have to meet the titratable acidity requirement indicated in the Codex standard (minimum of 0.9% titratable acidity).

Also, concentrated skim milk, non-fat dry milk, buttermilk, whey, lactose, lactalbumins, and lactoglobulins can be modified by partial or complete removal of lactose and/or minerals to increase the non-fat solids content of the food, provided that the ratio of protein to total non-fat solids of the food, and the protein efficiency ratio of all protein present shall not be decreased as a result of adding such ingredients. Nutritive carbohydrate sweeteners such as sugar (sucrose), beet or cane; invert sugar (in paste or syrup form); brown sugar; refiner's syrup; molasses (other than blackstrap); high-fructose corn syrup; fructose; fructose syrup; maltose; maltose syrup, dried maltose syrup; malt extract, dried malt extract; malt syrup, honey; and maple sugar can be used. Flavoring ingredients, color additives, and stabilizers can also be used.

16.2.3 China

16.2.3.1 Description

The following terms and definitions are adopted in the Standard. Yogurt is a product made of raw cow milk and goat milk or dry milk through a procedure of pasteurization and fermentation by inoculating *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Modified yogurt is a product made of over 80% raw cow milk and goat milk or dry milk through the procedures of pasteurization and fermentation by inoculating S*treptococcus thermophilus* and *Lactobacillus bulgaricus* with or without adding food additives, nutrition fortifiers and cereals, etc., before or after fermentation (China National Dairy Standard GB2746-1999).

16.2.3.2 Composition

Fermented milk is a product made of raw cow milk and goat milk or dry milk by decreasing the pH value after pasteurization and fermentation. Modified fermented milk is a product made of 80% raw cow milk and goat milk or dry milk by decreasing the pH value after pasteurization and fermentation and inoculating *Streptococcus thermophilus* and *Lactobacillus bulgaricus* with or without adding food additives, nutrition fortifiers and cereals, etc., before or after fermentation. The products previously mentioned will contain active lactobacilli within the quality assurance period. If the fermented product is subject to heat treatment, the index of viable count is no longer required. Various parameters and standards are listed in Table 16.7.

16.2.4 Japan

16.2.4.1 Description

Fermented milk means products that are obtained by fermenting milk, or milk products containing an equal or greater amount of milk solids-not-fat with lactic acid bacteria or yeast and then forming a paste, liquid, or frozen product (Ministry of Health and Welfare Ordinance No. 52).

16.2.4.2 Composition

Composition of fermented milk requires milk solids-not-fat content minimum 8.0%; lactic acid bacteria count or yeast count (per mL) minimum 10 000 000 cfu; coliforms negative; raw water for fermented milk shall be potable water. Raw materials for fermented milk (excluding lactic acid bacteria, yeast, fermented milk, and fermented milk drink) shall either be pasteurized by heating at 62°C for 30 min or by heating by a method having an equal or greater pasteurization effect.

16.2.5 Korea

16.2.5.1 Description

Fermented milk means a milk product obtained by fermentation of milk and/or products with lactic acid bacteria or yeast. The product can be added to other food or food additives sanitarily (Livestock Product Processing Control Act 2.1.4).

16.2.5.2 Composition

Classification of fermented milk is by milk solids-not-fat and live microorganisms. When the level of milk solids-not-fat is more than 3%, products are classified as fermented milk (yogurt beverage); when milk solids-not-fat is more than 8%, products are classified as cultured milk (yogurt). Composition requirements for various fermented milks are summarized in Table 16.8.

Table 16.7. Parameters and standards for fermented milk in China.

Item		Specification	
		Yogurt, fermented milk	Modified yogurt Modified fermented milk
Sensory indices	Color	Consistency in color: white or yellowish	Presents with its original unique color
	Taste and flavor	Presents with its original unique taste and flavor	Presents with its original unique taste and flavor
	Texture	Product has its own unique texture	Product has its own unique texture
Physicochemical indices	Fat (g/100 g)	≥3.1	≥2.5
	Non-fat solids (g/100 g)	≥8.1	—
	Protein (g/100 g)	≥2.9	≥2.3
	Acidity (°T)	≥70	≥70
Microorganism index	Coliform	n = 5, c = 2, m = 1 cfu/g, M = 5 cfu/g	
	Staphylococcus aureus	n = 5, c = 0, m = 0 cfu/25 g	
	Salmonella	n = 5, c = 0, m = 0 cfu/25 g	
	Yeast (cfu/g)	$\leq 10^2$	
	Fungi (cfu/g)	≤30	
	Lactobacillus (cfu/g)	$\geq 10^6$	

n, sample size; c, acceptance number; m, satisfactory standard; M, acceptability threshold.
Source: based on data from China-National Dairy Standard GB2746-1999 (http://www.procedurallaw.cn).

Table 16.8. Korean composition standards for various fermented milks.

Type	Regulation	Milk fat	Milk solids-not-fat	Live microorganism	Coliform
Fermented milk	It has own color	min. 3.0%	—	min. 10^7 cfu/mL	n = 5
Cultured milk	and flavor and	min. 8.0%	—	min. 10^8 cfu/mL	c = 1
Cream	no off-flavor	min. 3.0%	min. 8.0%	min. 10^7 cfu/mL	m = 0
Cultured cream		min. 8.0%	min. 8.0%	min. 10^8 cfu/mL	M = 10
Cultured buttermilk		min. 8.0%	min. 1.5%	min. 10^7 cfu/mL	

n, sample size; c, acceptance number; m, satisfactory standard; M, acceptability threshold.
Source: based on data from Korea-Livestock Product Processing Control Act 2.1.4; Korean Ministry for Food, Agriculture, Forestry and Fisheries (http://www.mifaff.go.kr).

16.3 HEALTH BENEFITS OF FERMENTED MILK PRODUCTS

16.3.1 Nutritional benefits

Many studies have shown that fermentation with lactobacilli improves the nutritional value of food products by increasing the quantity, availability, digestibility, and assimilability of nutrients. Yogurt contains higher levels of free amino acids than milk because of proteolysis by the yogurt microbiota. The proteins of acidophilus milk, bifidus milk, yogurt, and buttermilk are more digestible than the proteins in unfermented milk (Hargrove & Alford, 1978).

While many lactobacilli require vitamin B for growth, cultures of these organisms are capable of synthesizing certain vitamins. Fermentation has been reported to increase the folic acid content in a variety of products, including yogurt, bifidus milk, and kefir (Deeth & Tamime, 1981; Alm, 1982). There have also been studies showing an increase in niacin and riboflavin in yogurt, vitamin B_{12} in Cottage cheese, and vitamin B_6 in Cheddar cheese. In

addition, cultured dairy products contain higher levels of the vitamin B group, in particular folic acid, than their counterparts prepared by direct acidification. The thiamin and riboflavin content has also been shown to be higher in *Lactobacillus*-fermented products (McDonough *et al*., 1983). The bioavailability of copper, calcium, iron, zinc, manganese, and phosphorus is higher in yogurt than in milk. Consistent with these findings, the growth rate differential between rats fed yogurt and those fed milk can be reduced by supplementation of the milk with minerals, especially copper, iron, zinc, and manganese.

16.3.2 Diarrheal disease

The most extensive medical literature about probiotics and fermented milk is in the area of diarrheal diseases. The treatment and prevention can be further categorized by etiological agents or by the type of disease.

Diarrhea occurs in about 20% of patients who receive antibiotics. Antibiotic-associated diarrhea (AAD) results from a microbial imbalance that leads to a decrease in the fermentation capacity of the colon. Invasion with *Clostridium difficile*, *Klebsiella oxytoca*, and *Clostridium septicum* are the primary causes of AAD (Salminen *et al*., 1998; Ziemer & Gibson, 1998). Studies have shown that oral administration of *Saccharomyces boulardii* can decrease the risk of AAD (Surawics *et al*., 1989). Other studies have shown that *S. boulardii* (Adams *et al*., 1977), *Enterococcus faecium* SF68, and *Lactobacillus rhamnosus* GG can shorten the duration of AAD (Marteau *et al*., 2001).

Visitors from countries with temperate climates to areas with tropical or subtropical climates experience a high incidence of diarrhea. The incidence rate often approaches 50%. Some studies have shown that *S. boulardii* and *Lactobacillus rhamnosus* GG significantly prevent traveler's diarrhea. Several trials have demonstrated that some fermented products act to prevent diarrhea in children (Saavedra *et al*., 1994). In addition, feeding *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants was shown to significantly reduce the risk of diarrhea and the shedding of rotavirus.

Disturbances in the normal intestinal microbial community structure can result in the proliferation of pathogens. Acute inflammatory reactions cause diarrhea and sometimes vomiting, and can be associated with a number of bacteria and viruses, including *Escherichia coli*, *Campylobacter* spp., *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Salmonella* spp., *Shigella* spp., *Yersinia* spp., and protozoa, especially *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium parvum*. Bacteria can also be linked to more chronic diseases in the colon. For example, *Clostridium difficile* has been targeted as the primary causative agent of pseudomembranous colitis (Salminen *et al*., 1998). Several effects of probiotics against diarrhea are summarized in Table 16.9.

16.3.3 Immune regulation

Most extensive studies directed at probiotic modulation of the immune response to food allergens has been done with *Lactobacillus rhamnosus* GG in preventing and treating atopic eczema. In a study of 27 infants with atopic eczema, the children were randomized into three groups and given *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* subsp. *lactis* or placebo (Isolauri *et al*., 2000). After 2 months the clinical score for the severity and extent of the eczema indicated a significant improvement in the skin condition of the infants fed the probiotics.

The gastrointestinal tract functions as a barrier against antigens from microorganisms and food (Isolauri *et al*., 2001). Among the possible mechanisms of probiotic therapy is promotion of a non-immunological gut defense barrier, which includes the normalization of increased intestinal permeability and altered gut microecology. Another possible mechanism is to improve the intestinal immunological barrier, particularly through IgA responses and alleviation of gut inflammatory responses, which produce a stabilizing effect. Many probiotic effects are mediated through immune regulation, particularly the balance of proinflammatory and anti-inflammatory cytokines (Isolauri *et al*., 2001).

In a human trial, administration of probiotic yogurt produced an increase in the production of interferon-γ (Halpern *et al*., 1991). In animal models, probiotics have been shown to stimulate the production of antibodies (local and systemic), enhance the activity of macrophages, increase interferon-γ levels, and increase the concentration of natural killer cells (Fooks *et al*., 1999).

Inflammatory bowel disease (ulcerative colitis and Crohn's disease) is related to the intestinal microbiota. Symptoms of inflammatory bowel disease include disturbance in bowel habit and mucosal inflammation. In the intestine of people with inflammatory bowel disease, the numbers of *Lactobacillus* and *Bifidobacterium* are lower and those of coccoids and anaerobes are higher. Probiotics do not cure the disease, but once patients are in remission through treatment with corticosteroids, some probiotics can prolong the remission period and reduce the incidences of relapse and the use of corticosteroids (Shah, 2007). This improves the quality of life of patients. Probiotic microorganisms and inflammatory bowel disease are summarized in Table 16.9.

16.3.4 Prevention of osteoporosis

Dairy products such as milk have been proposed as a nutritional food that aids in the prevention of osteoporosis because of its bioavailable calcium content (Silverwood, 2003). Lactoferrin is an iron-binding glycoprotein and a

Table 16.9. Probiotic microorganisms and inflammatory bowel disease.

Microorganism	Probiotic function	Reference
Enterococcus faecium	Decreased duration of acute diarrhea from gastroenteritis	Marteau *et al*. (2001)
Lactobacillus acidophilus	Significant decrease in diarrhea in patients receiving pelvic irradiation	Marteau *et al*. (2001)
Lactobacillus plantarum	Especially effective in reducing inflammation in inflammatory bowel, e.g., enterocolitis in rats, small bowel bacterial overgrowth in children, pouchitis. Reduced pain and constipation in irritable bowel syndrome. Reduced bloating, flatulence, and pain in irritable bowel syndrome in controlled trial	Schultz & Sartor (2000), Vanderhoof (2000)
Lactobacillus reuteri	Shortens the duration of acute gastroenteritis Shortens acute diarrhea	Marteau *et al*. (2001) Shornikova *et al*. (1997a, b)
Saccharomyces boulardii	Reduced recurrence of *Clostridium difficile* diarrhea. Effects on *Clostridium difficile* and *Klebsiella oxytoca* resulted in decreased risk and/or shortened duration of antibiotic-associated diarrhea. Shortened the duration of acute gastroenteritis. Decreased only functional diarrhea, but not any other symptoms of irritable bowel syndrome	Pochapin (2000)
Bifidobacterium bifidum, *Streptococcus thermophilus*	Reduction in rotavirus shedding and episodes of diarrhea in children in hospital	Saavedra *et al*. (1994)
Enterococcus faecium PR88	Symptomatic improvement in 19 of 28 patients with high-volume diarrhea caused by food intolerance for 12 weeks and a significant decrease in fecal weight	Hunter *et al*. (1996)
Saccharomyces boulardii *Lactobacillus rhamnosus* GG	Helps prevent antibiotic-associated diarrhea *Saccharomyces boulardii* appears useful for *Clostridium difficile* disease	McFarland (2006)

non-enzymatic antioxidant found in the whey fraction of milk (Marshall, 2004). Lactoferrin functions in bone metabolism by promoting osteoblast cell growth and reducing osteoclast differentiation and resorption activity (Lorget *et al*., 2002; Cornish, 2004).

In *in vitro* and animal studies, milk basic protein (MBP, Snow Brand Milk Products, Japan) had the ability to stimulate proliferation and differentiation of osteoblastic cells as well as suppress bone resorption (Takada, 1997; Toba *et al*., 2000). MBP is prepared from fractionated whey using a cation-exchange resin. *In vivo* studies on rats determined that both whey protein and fractionated whey protein had the ability to increase femoral bone strength in ovariectomized rats (Takada *et al*., 1997; Kato *et al*., 2000).

In a recent report, a milk product fermented by *Lactobacillus casei* 393 proliferated osteoblastic MC3T3-E1 cells and attenuated the reduction of bone strength that occurred in response to ovariectomy. These effects were related to increased organic matter and calcium contents in the bone. Fermented milk product (1%) increased the bone mineral density and bone breaking force of femurs in supplemented rats compared with ovariectomized rats (Fig. 16.1).

16.3.5 Cholesterol reduction

For the last several decades, the concern with mechanisms of cholesterol reduction by probiotics has been growing. As fermented milk containing probiotics was first demonstrated to exhibit hypocholesterolemic effects in humans as early as 1963 (Shaper *et al*., 1963; Mann & Spoerry, 1974), numerous studies have been conducted and as a result it was shown that some species of lactic acid bacteria, especially lactobacilli, can lower total cholesterol and low-density lipoprotein (LDL) cholesterol (Anderson & Gilliland, 1999; Sanders, 2000; Liong & Shah, 2005).

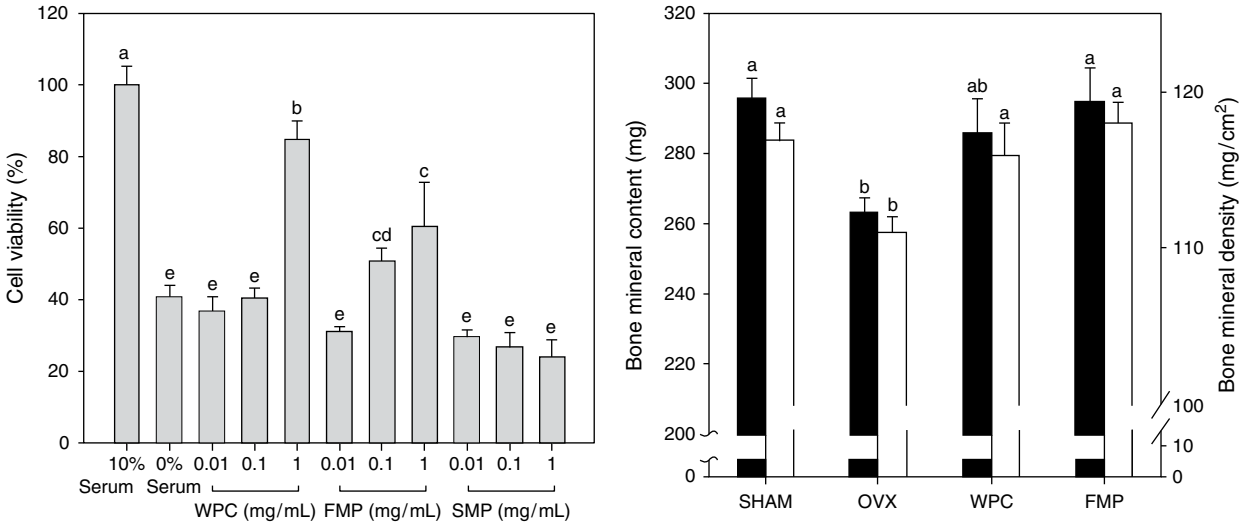


Figure 16.1. Effects of whey protein concentrate (WPC), fermented milk product (FMP), and skim milk powder (SMP) on MC3T3-E1 cell viability (%), bone mineral content (mg), and bone mineral density (mg/cm^2). Serum, blood serum (a component of blood which is collected after coagulation); SHAM, sham-operated group (control group in laboratory experiment); OVX, ovariectomized rat. Solid bars represent bone mineral content; open bars represent bone mineral density. The different letters above the bars indicate significant differences ($P < 0.05$). The differences among treatments were tested by Duncan's new multiple range test. Reproduced from Kim *et al.* (2009), with permission of Elsevier.

Lee *et al*. (2010) investigated factors involved in serum cholesterol reduction by *Lactobacillus acidophilus* using a mutant that had decreased cholesterol reduction ability. In the colorimetric assay, *Lactobacillus acidophilus* A4 and most of its 600 mutants were found to reduce cholesterol in the media by approximately 50%; however, one colony, BA9, reduced the amount of cholesterol in the media by only 7.7%. This difference was confirmed by gas chromatography using a flame ionization detector (FID), which showed greatly attenuated cholesterol reduction in the BA9 mutant (Fig. 16.2a). From these two analyses, it was clear that the BA9 mutant had an impaired ability to reduce cholesterol in the medium. There were no differences in biomass or growth rates in the wild-type and BA9 strains. To identify the genes responsible for cholesterol reduction in *Lactobacillus acidophilus* A4, the transposon insertion sites of the BA9 mutant were mapped, which had reduced cholesterol-lowering activity. Then the impact of the specific protein encoded by the *ccpA* gene was explored and differences in protein expression profiles between the *Lactobacillus acidophilus* A4 parent strain and the BA9 mutant were analyzed using two-dimensional gel electrophoresis and chemically assisted fragmentation–matrix-assisted laser desorption/ionization. In addition, an *in vivo* study was conducted to examine differences in serum cholesterol reduction in rats that ate the mutant or wild-type bacteria (Fig. 16.2b). These results led to the conclusion that the modified genetic and proteomic factors identified in the *in vitro* studies may be responsible for the lowered cholesterol levels and/or modulated lipid metabolism observed *in vivo*. However, further studies are needed to verify this conclusion.

16.3.6 Cancer prevention

Mutagenic compounds commonly found in the Western meat-rich diet are physical or chemical agents that change the genetic material, usually DNA, of an organism and thus increase the frequency of mutations above the natural background level. As many mutations cause cancer, mutagens are therefore also likely to be carcinogens. They also cause changes to the DNA that can affect the transcription and replication of the DNA, which in severe cases can lead to cell death. The mutagen produces mutations in the DNA, and deleterious mutation can result in aberrant, impaired or loss of function for a particular gene, and accumulation of mutations may lead to cancer. These mutagenic compound bind to the intestinal lactic acid bacteria *in vitro* and binding correlates well with the reduction in mutagenicity observed after exposure to the bacterial strains (Zhang & Yoshiyuki, 1991; Lankaputhra & Shah, 1998). Binding correlates well

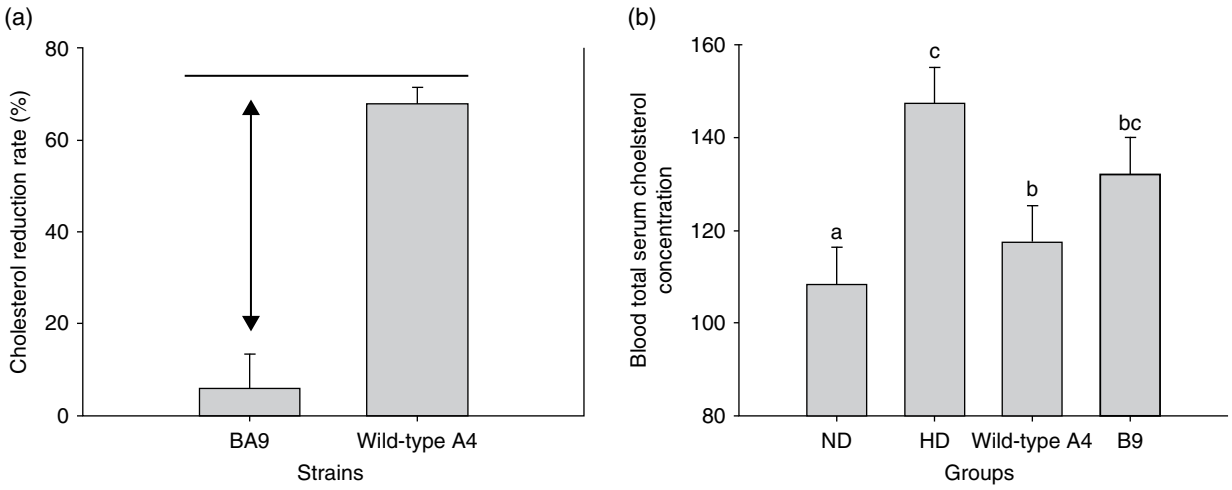


Figure 16.2. (a) GC/FID analysis of cholesterol removal by *Lactobacillus acidophilus* A4 and the BA9 mutant. (b) Total serum cholesterol concentration of rats fed by each experimental diet at week 5. ND, normal diet; HD, hypercholesterolemic; Wild-type A4, hypercholesterolemic diet + *Lactobacillus acidophilus* A4; B9, hypercholesterolemic diet + mutant BA9 of *Lactobacillus acidophilus* A4. The different letters on diagrams indicate significant differences ($P < 0.05$). The differences among treatments were tested by Duncan's new multiple range test. Reproduced from Lee *et al.* (2010), with permission of the American Society for Microbiology.

with the reduction in mutagenicity observed after exposure of the heterocyclic amines found in cooked, and especially burned, meat to the bacterial strains. The binding appears to be a physical phenomenon, mostly due to a cation exchange mechanism and it has been suggested that cell wall peptidoglycans and polysaccharides are the two most important elements responsible for the binding (Zhang & Yoshiyuki, 1991). However, there is a danger in extrapolating these results to health claims for humans, as the reversibility of mutagen binding to cultures *in vivo* is unknown. Furthermore, the biologically significant levels of mutagens and of lactic acid bacteria in the human system are unknown. Lactic acid bacteria or a soluble compound produced by the bacteria may interact directly with tumor cells in culture and inhibit their growth (Reddy & Lalwai, 1983).

Diets high in animal protein and fat appear to increase susceptibility to colon cancer, apparently through conversion of procarcinogens to carcinogens by the intestinal microbiota. Fats and fried foods have also been implicated in cancers of the breast, prostate, and pancreas. The consumption of milk has been negatively correlated with the incidence of gastric cancer and has been postulated to play an important role in prevention of human stomach cancer caused by alkylating agents (Yano, 1979). On the other hand, milk consumption has also been positively correlated with the incidence of colon, prostate, and breast cancer (Gaskill *et al*., 1979) and has been attributed to the increased consumption of fat, modification of the intestinal flora by milk components, ingestion of milk hormones, and presence of an oncogenic virus or other contaminants in milk.

It is hypothesized that gut microbiota, through the production of carcinogens and tumor promoters, are involved in the etiology of colon/rectal cancer. There is some evidence that probiotics can interfere at various stages of the cancer process, such as prevention of DNA damage in the colon by live bacteria (Pool-Zobel *et al*., 1996), suppression of preneoplastic changes in the colon (Rowland *et al*., 1998), and suppression of colon tumors in animals (Mcintosh *et al*., 1999).

Several lactic acid bacteria may help prevent initiation of cancer (Table 16.10). It appears that lactic acid bacteria can reduce the levels of colon enzymes that convert procarcinogens to carcinogens. Specifically, lactic acid bacteria can reduce levels of the enzymes β-glucuronidase, nitroreductase, and azo-reductase. Lactic acid bacteria may also be involved in the direct reduction of procarcinogens (Kumar *et al*., 2010).

Feeding of *Lactobacillus acidophilus* and *Bifidobacterium longum* suppressed the formation of aberrant crypt foci (ACF) and tumor incidence induced by azoxymethane (Arimochi *et al*., 1997; Challa *et al*., 1997; Rowland *et al*., 1998). In addition, it has been reported that colonization of bacteria with an ability to produce genotoxic compounds and high β-glucuronidase activity enhances progression of ACF induced by 1,2-dimethylhydrazine (DMH) in rats, and that additional colonization with *Bifidobacterium breve* reduced the number of ACF with

Table 16.10. Examples of carcinogens, their biomarkers and potential probiotics that reduce their effects.

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Table 16.10 (*Continued*)

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four or more crypts/focus and crypt multiplicity that are reliable predictors of malignancy (Onoue *et al*., 1997).

Reddy and Rivenson (1993) reported that lyophilized cultures of *Bifidobacterium longum* administered in the diet to rats inhibited liver, colon, and mammary tumors induced by the food mutagen 2-amino-3-methyl-3H-imidazo[4,5-*f*]quinoline. Goldin and Gorbach (1980) showed that dietary supplements of *Lactobacillus acidophilus* not only suppressed the incidence of DMH-induced colon carcinogenesis but also increased the latency period in rats. Feeding of fermented milk increased the survival rate of rats with chemically induced colon cancer (Shackelford *et al*., 1983).

Studies on the effect of probiotic consumption on cancer appear to be promising (Friend & Sahani, 1984). Animal and *in vitro* studies indicate that probiotic bacteria may reduce colon cancer risk by reducing the incidence and number of tumors. One clinical study showed an increased recurrence-free period in subjects with bladder cancer (Aso & Akazan, 1992). However, results are too preliminary to develop specific recommendations on probiotic consumption for preventing cancer in humans.

16.4 FUTURE ASPECTS

Introduction of new fermented food products containing beneficial bacteria will emerge, such as cereals, energy bars, cheese, juices, disease-specific medicinal foods, and infant foods. The identity of beneficial bacteria in fermented foods will serve to accelerate the development and availability of a range of new healthy fermented food products. Fermented foods have been part of the human diet for centuries, and may become important in the diets of future space travelers.

New strains will be identified and food products will be developed to fulfill the needs of specific consumers. An increased understanding of the viability of probiotic bacteria, and interactions between gut microbiota, diet, and the host will open up new possibilities for producing new ingredients for nutritionally optimized foods, which promote consumer health through microbial activities in the gut.

Gene technology is also playing an important role in the development of new strains, where gene sequencing has been used to provide a better understanding of the mechanisms and functionality of probiotics, which is required for improving current fermented foods. In addition, industry-based probiotic research will focus on increasing the shelf-life of fermented foods and increasing the survival rate of probiotics within the intestinal tract by introducing new stress tolerance strains and improved handling and packaging procedures to ensure that the desired health benefits are delivered to the consumer.

The health benefits of fermented functional foods are expressed either directly through the interaction of ingested live microorganisms (bacteria or yeast) or indirectly through the ingestion of microbial metabolites produced during the fermentation process (biogenic effect). As more is learned about the role of microorganisms in human nutrition, immune function, and disease resistance, the number of fermented products available on the market will increase. New research supports the effectiveness of fermented food products or probiotics for the treatment and prevention of infectious disease and AAD. Fermented food therapy has been applied to a wide range of health concerns, such as immune system disorders and treatment of gastrointestinal problems. Fermented foods with impact on health will be an important functional ingredient in the future (Tamang & Kailasapathy, 2010).

This chapter has outlined the early history of fermented milk, nutritional benefits, standards and regulations of current products, and the evidence supporting health applications. The chapter has also attempted to predict future developments of probiotics based on the latest technological advances in the field of microbial genetics. These future developments will provide new applications for fermented milk in the fight against the multiple diseases and disorders that afflict humankind.

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17
Cheese Science and Technology

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17.1 INTRODUCTION

Cheese is a group of fermented dairy products which evolved in the 'Fertile Crescent' between the Tigris and Euphrates rivers about 8000 years ago, during the so-called 'Agricultural Revolution'. Approximately 17 million tonnes (Mt) of cheese are produced throughout the world per annum in a great diversity of flavours, textures and forms; more than 1000 varieties of cheese are recorded, most of which are produced locally and are of only minor importance. The principal 'international' varieties are Cheddar (~2 Mt per annum), Mozzarella (pizza, ~1.5 Mt per annum), Gouda, Emmental, Grana types, Camembert and Quark. Liquid milk and cheese are, quantitatively, the most important dairy products; they account for about 40% and 30% of total milk production, respectively. The principal cheese-producing regions are Europe, North and South America, Australia and New Zealand; the proportion of milk used for cheese is as high as 70–90% in some European countries, for example Italy, France, Germany and Denmark (International Dairy Federation, 2008). Cheese production has increased by about 2% per annum over several decades because of its positive nutritional image, convenience, functionality and price.

Essentially, cheese curd is produced by coagulating the casein fraction of milk protein by acidification to about pH 4.6 at approximately 30°C or by limited proteolysis using rennets; rennet-coagulated cheese represents about 75% of total cheese production. Some minor varieties are produced by coagulating the whey proteins by heating at 90°C.

The production of all varieties of cheese involves a generally similar protocol, various steps of which are modified to give a product with the desired characteristics. The general protocol for the manufacture of acid-coagulated cheese is summarised in Fig. 17.1a and of rennet-coagulated cheese in Fig. 17.1b.

There is a very extensive literature on cheese science and technology; major textbooks include Davis (1965, 1967), Kosikowski and Mistry (1997), Robinson and Wilbey (1998), Eck and Gilles (2000), Fox *et al*. (2000, 2004) and Law and Tamime (2010).

17.2 SELECTION AND TREATMENT OF MILK

The composition of cheese is strongly influenced by the composition of the milk, especially the content of fat, protein, calcium and pH value. The constituents and composition of milk are influenced by several factors, including species, breed, individual animal variations, nutritional status, health and stage of lactation of the milk-producing animals (Walstra & Jenness, 1984; Wong *et al*. 1988; Fox & McSweeney, 1998, 2003, 2006; Walstra *et al*., 1999, 2005; McSweeney & Fox, 2009, 2013; Guinee & O'Brien, 2010; Fuquay *et al*., 2011). Owing to major compositional abnormalities, milk from cows in the very early or late stages of lactation and those suffering from mastitis should be excluded. Somatic cells (leucocytes) are of three main types: lympocytes, phagocytes and mammary gland epithelial cells. Lymphocytes function in humoral and cell-mediated immunity while phagocytes, of which there are two types

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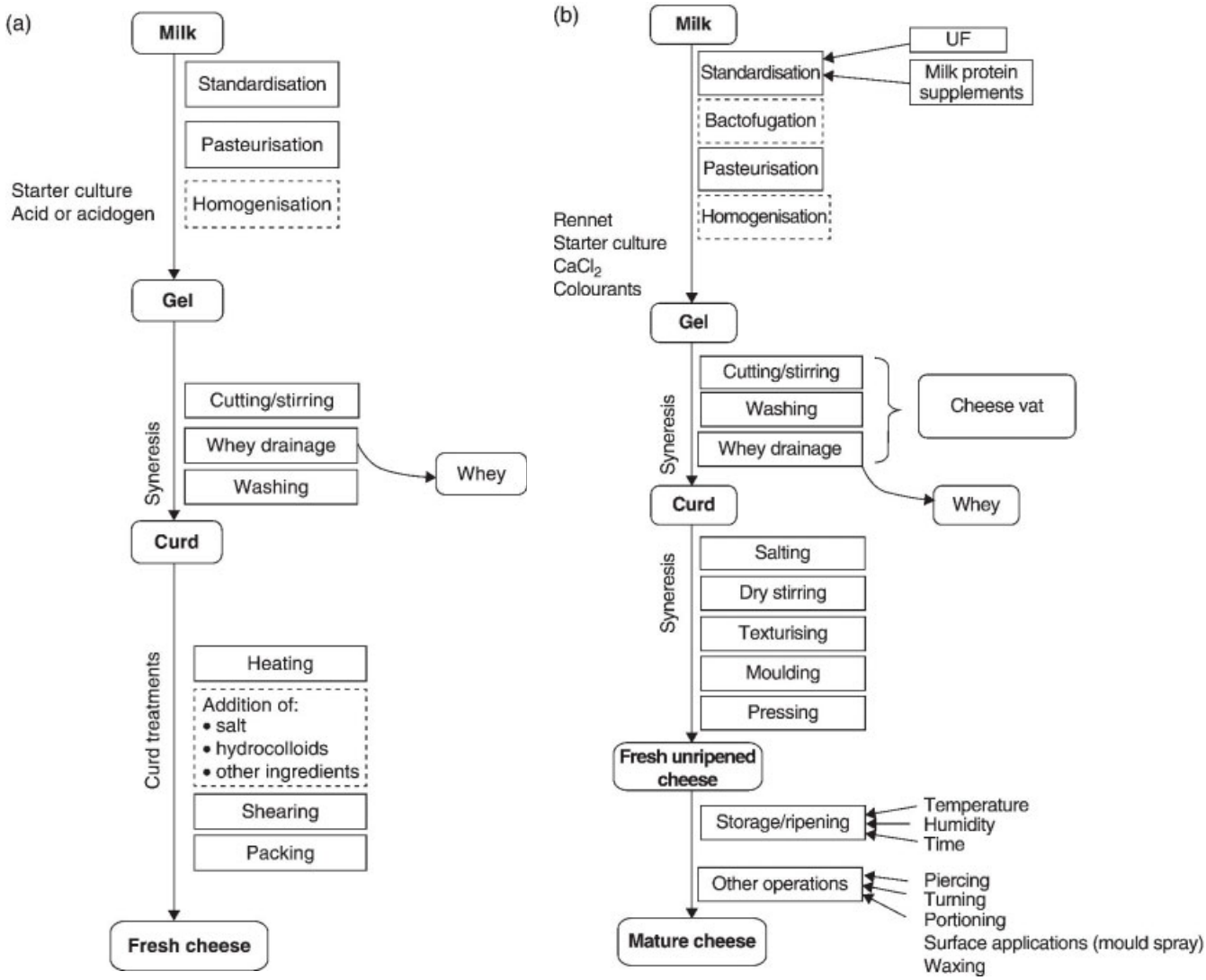


Figure 17.1. (a) General protocol for the manufacture of acid curd cheese. (b) General protocol for the manufacture of rennet curd cheese. UF, ultrafiltration.

(polymorphonuclear leucocytes and macrophages), ingest and kill pathogenic microorganisms which invade the mammary gland. Somatic cells in milk increase in response to bacterial infection of the udder. The somatic cell count (SCC) increases from less than 100×10^3 cells/mL in normal milk from healthy animals in mid-lactation to 5000×10^3 cells/mL in milk from cows suffering from clinical mastitis. Elevated SCC in milk is associated with marked changes in the concentrations of milk constituents, degree of hydrolysis of caseins and cheesemaking properties (Cooney *et al*., 2000). A high SCC ($>300 \times 10^3$ to 1000×10^3 cells/mL) has several adverse effects for cheese manufacture:

- a reduction in the levels of fat and casein in milk, and in casein as a percentage of true protein;
- impaired milk coagulation and curd-forming capacity;
- reduced cheese yield, associated with lower recovery of casein from milk to cheese; and
- higher cheese moisture, and rates of primary and secondary proteolysis during maturation.

The negative impact of SCC on cheese yield is attributed to the lower casein content and hydrolysis of α_{s1}- and β-caseins to products (γ-caseins, proteose peptones and other peptides) that are soluble in the serum and are not recovered in the cheese. Such proteolysis ensues from the elevated proteolytic activity of plasmin (and probably other proteinases), plasminogen and plasminogen activators in the milk that parallels increasing SCC (Cooney *et al*., 2000). The decrease in the intact casein level causes a marked deterioration in rennet gelation properties, impaired syneresis and a reduction in cheese yield.

All the lactoproteins of cow, buffalo, sheep and goat exhibit genetic polymorphism (Ng-Kwai-Hang & Grosclaude, 2003), some of which have a significant effect on cheese yield and quality, and there is increasing interest in breeding for desirable polymorphs, especially in the case of sheep and goats. In bovine milk, the genetic variant of κ-casein has a major influence on the cheesemaking properties of milk; κ-casein BB variant gives superior rennet coagulation characteristics, fat recovery from milk to cheese and cheese yield capacity than milk containing κ-casein AB, which, in turn, is superior to milk containing AA or AE genotypes. Reported increases in moisture-adjusted cheese yield with the κ-casein BB variant, compared with κ-casein AA, range from about 3% to 8%, depending on milk composition and cheese type. Generally, κ-casein AB has been found to exhibit rennet coagulation and cheese-yielding characteristics that are intermediate between those of κ-casein AA and BB. The superior rennet coagulation and cheese-yielding characteristics of κ-casein BB compared with the AA variant appear to be related to its higher casein content, higher level of κ-casein as a percentage of total casein, smaller micelles and lower negative charge. These properties are conducive to a higher degree of casein aggregation and a more compact arrangement of the *para*-casein micelles, which in turn favour more numerous intermicellar bonds during gel formation (Walsh *et al*., 1998a, b; Ng-Kwai-Hang & Grosclaude, 2003).

Cheese milk should be free of chemical taints and free fatty acids, which cause off-flavours in the cheese, and antibiotics, which inhibit bacterial cultures.

17.2.1 Milk of different species

A major cause of variation in the characteristics of cheese is the species of dairy animal from which the milk is obtained. The principal dairying species are cattle, water buffalo, goats and sheep, which produce approximately 84%, 12%, 2% and 1.5% of commercial milk, respectively. Goats and sheep are significant dairy animals in certain regions, for example around the Mediterranean, where their milk is used mainly for the production of fermented milks and cheese. Many world-famous cheeses are produced from sheeps' milk, for example Roquefort, Feta, Pecorino Romano and Manchego; traditional Mozzarella (Mozzarella di buffalo) is made from buffalo milk. There are very significant interspecies differences in the composition and physicochemical properties of milk which are reflected in the characteristics of cheese produced therefrom. There are also significant differences in milk composition between breeds of cattle that influence cheese quality. The milk of yak is important in China and Tibet and a little is used to produce artisanal cheese (Park & Haenlein, 2006; Wiener, 2011). A little cheese is also produced from reindeer milk in the Artic regions of Russia and Scandinavia (Park & Haenlein, 2006). The milk of camel, horse and donkey yield a very weak, or no, gel and cannot be used for cheese production. These non-bovine milks are described in Chapters 23–30 and in several articles in Fuquay *et al*. (2011). The composition and properties of buffalo milk were reviewed comprehensively by Abd El-Salam and El-Shabini (2011). The poor rennetability of equine and asinine milk has not been fully described but a low concentration of casein, and of κ-casein in particular, are probably causal factors, although a high $[Ca^{2+}]$ should promote coagulability (Uniacke-Lowe, 2011). Camel milk produces only a sloppy gel with calf chymosin and cannot be used for cheesemaking. However, satisfactory cheese can be made from camel milk using camel chymosin, which is now available from genetically engineered *Aspergillus niger* (Bansal *et al*., 2009; Farah, 2011).

The milk for cheese should be of good microbiological quality, as contaminating bacteria are concentrated in the curd and may cause defects or public health problems. However, most cheese milk is pasteurised or subjected to one

or more of the treatments described in section 17.2.3, to render it free of pathogenic, food poisoning and spoilage bacteria.

17.2.2 Standardisation of milk composition

The composition of the principal varieties of cheese is prescribed in 'Standards of identity' (Hickey, 2011) with respect to moisture and fat in dry-matter, which in effect defines a certain fat–protein ratio. The moisture content of cheese, and hence the level of fat and protein, is determined mainly by the manufacturing protocol but the fat–protein ratio in cheese is determined mainly by the fat–casein ratio in the milk. Depending on the ratio required, it can be modified by removing some fat by natural creaming or centrifugation, adding skimmed milk, cream, milk powder, evaporated milk or ultrafltration retentate; such additions also increase the total solids content of the milk and hence increase the yield of cheese curd per unit volume. The two extremes of compositional adjustment are the preparation of skimmed milk for cheeses such as Cottage and Quark or the preparation of cream for the production of Cream cheese.

Calcium plays an essential role in the coagulation of milk by rennet and in the subsequent processing of the coagulum; hence, it is common practice to add 0.01% $CaCl_2$ to cheese milk. $CaCl_2$ added to milk reacts with soluble phosphate to form colloidal calcium phosphate (CCP) with the release of H^+, and with glutamate and aspartate residues, thereby reducing the negative charge on the protein. The reduced pH, increased $[Ca^{2+}]$ (by 0.3 mmol/L) and increased CCP concentration reduce the rennet coagulation time, increase the firmness of the gel and improve the syneresis of the gel when cut; however, the increased level of calcium may increase brittleness and reduce the meltability of cheese.

The pH of milk is a critical factor in cheesemaking. The addition of 1.5–2% of a bulk starter culture to cheese milk, as practised traditionally, reduces its pH by about 0.1 units but starter concentrates (direct vat starters) have little or no direct acidifying effect. Previously, it was standard practice to add the starter to the milk 30–60 min before rennet addition. The objective of this operation, referred to as 'ripening', was to allow the starter bacteria to enter the exponential growth phase and become highly active during cheesemaking; ripening is not necessary with modern high-quality starters. Some acid is produced during ripening which favours rennet action and gel formation. However, ripening increases the risk of bacteriophage infection of the starter because phage become distributed throughout the liquid milk but are fixed in position after it has coagulated and therefore their destructive effect is localised and reduced. Although ripening may still be practised for some cheese varieties, it has been discontinued by large producers.

The pH of milk on reception at the dairy is higher today than previously owing to improved hygiene during milking and the widespread use of refrigeration at the farm and factory. In the absence of acid production by contaminating bacteria, the pH of milk increases slightly during storage due to the loss of CO_2 to the atmosphere. The natural pH of milk is about 6.7 but varies somewhat (e.g. it increases in late lactation and during mastitic infection).

As an alternative to ripening, the pre-acidification of milk by 0.1–0.2 pH units, either through the use of gluconic acid-δ-lactone (GDL) or by limited growth of a lactic acid starter (referred to as pre-maturation) followed by pasteurisation, is recommended and results in better and more uniform rennet coagulation characteristics and cheese quality.

17.2.3 Heat treatment of milk

Traditionally, cheese was made from raw milk, a practice which was almost universal until the 1940s. Although cheese made from raw milk develops a more intense flavour than that produced from pasteurised milk, the former is less consistent and poses a potential public health risk. When cheese was produced from fresh milk on farms or in small, local factories, the growth of contaminating microorganisms was minimal but as cheese factories became larger, storage of milk for longer periods became necessary and hence the microbiological quality of the milk deteriorated and varied. Thermisation (heating at ~65°C for 15 s) of cheese milk is fairly widely practised on receipt at the factory to reduce the microbial load and extend the storage period. For public health reasons, it became increasingly popular from the beginning of the twentieth century to pasteurise milk for liquid consumption. The pasteurisation of cheese milk became widespread about 1940, primarily for public health reasons, but also to provide a milk supply of more uniform bacteriological quality. Although a considerable amount of cheese is still produced from raw milk, on both an artisanal and factory scale, especially in southern Europe (including such famous varieties as Swiss Emmental, Gruyère Comte, Parmigiano Reggiano and Grana Padano), pasteurised milk is now generally used, especially in large factories. The curds for all the above cheeses are 'cooked' to about 55°C and remain at this temperature for quite a long time; consequently, these cheeses are phosphatase negative and hence are technically pasteurised; also they are ripened for a long period (>1.5 years for Parmigiano Reggiano) during which any surviving pathogens die off. However, high-moisture cheeses made from raw milk pose a potential health risk because they are cooked to a low temperature and are ripened for a short period during which the pH of many of them increases.

It is generally accepted that the flavour of raw milk cheese is different from, and stronger than, that of pasteurised milk

cheese due to the killing of desirable bacteria, for example non-starter lactic acid bacteria and undesirable bacteria. This collateral damage is unavoidable but the flavour of pasteurised milk cheese can be intensified and made to simulate raw milk cheese by the addition of selected lactobacilli to the cheese milk as an adjunct starter, a practice which is becoming increasingly common for Cheddar cheese. The use of an adjunct culture also offers the possibility of producing cheese with a flavour profile identifiable with a particular company (manufacturer or retailer). It is likely that the use of adjunct cultures will increase.

There are four alternatives to pasteurisation for reducing the number of microorganisms in milk, but it is important to realise that these methods may not produce pathogen-free milk:

1. Treatment with H_2O_2: not practised in developed dairying countries.
2. Activation of the lactoperoxidase–H_2O_2–thiocyanate system: very limited or no application in the cheese industry.
3. Bactofugation: frequently used to remove clostridial spores as an alternative to the use of nitrate to prevent late gas blowing in cheese.
4. Microfltration: very effective for removing bacteria and spores from milk but not yet widely practised in the cheese industry for this purpose.

Clostridium tyrobutyricum is of particular importance in the manufacture of many/most cheese varieties, because it catabolises lactic acid, producing CO_2, H_2 and butyric acid, and causing late gas blowing and off-flavours. Cheddar-type cheeses do not suffer from this problem, because the rapid decrease in pH to below 5.4 and dry salting prevent the growth of *Cl. tyrobutyricum*. For susceptible cheeses, the problem is controlled by minimising contamination of the milk, including banning the feeding of silage to the dairy cows, bactofugation of the milk, adding $NaNO_3$ to the milk which is reduced to $NaNO_2$, and addition of lysozyme to the milk (lysozyme is more expensive than $NaNO_3$).

17.2.4 Cheese colour

The principal pigments in milk are carotenoids which are obtained from the animal's diet, especially from fresh grass and clover. Cattle transfer carotenoids to adipose tissue and milk but goats, sheep and buffaloes do not. Therefore, bovine milk fat and high-fat products, including cheese, are yellow to an extent dependent on the carotenoid content of the animal's diet, whereas their counterparts made from sheep, goat or buffalo milk are very white in comparison. The yellowish colour of dairy products produced from cows' milk may make them less acceptable than products produced from sheep, goats or buffalo milk in regions where the latter are traditional. The carotenoids in bovine milk can be bleached by treatment with H_2O_2 or benzoyl peroxide or masked by chlorophyll or titanium oxide, if these additives are permitted.

At the other end of the spectrum are individuals who prefer highly coloured cheese, which is usually achieved by adding annatto, extracted from the seeds of *Bixa orellana*, a native of Brazil, which contains two apocarotenoid pigments, bixin and norbixin. Alternatively, synthetic or natural carotenoids may be used. The supplementation of milk for English-type cheeses, for example Cheddar and Gloucester, with carotenoid pigments is common (some consumers believe that the flavour of 'red' Cheddar is superior to that of 'white' Cheddar) but the practice is rare, or non-existent, for other varieties.

17.3 CONVERSION OF MILK TO CHEESE CURD

After the milk has been standardised and pasteurised or otherwise treated, its temperature is adjusted to a value in the range 30–35°C, depending on the variety, and transferred to vats (or kettles), which vary in shape (hemispherical, rectangular or vertical or horizontal cylinders), may be open or closed and may range in size from a few hundred litres to 30 000 L or more (Kosikowski & Mistry, 1997; Bennett & Johnston, 2004), where it is converted to cheese curd by a process which involves three basic operations: acidification, coagulation and dehydration.

17.3.1 Acidification and starter cultures

Acidification is usually achieved through the *in situ* production of lactic acid by the fermentation of lactose by lactic acid bacteria (LAB). Initially, the endogenous milk microflora was relied upon to produce acid but since this was variable, the rate and extent of acidification were slow and variable, resulting in cheese of variable quality. Cultures of LAB (starters) for cheesemaking were introduced about 120 years ago and since then have been improved and refined progressively. The acidification of curd for some artisanal cheeses still relies on the endogenous microflora but a starter is now generally used for most varieties made from either raw or pasteurised milk. Essentially, three types of starter are used (Fox *et al*., 2004):

1. '*Slop-back' cultures*: whey from one day's cheese production is incubated overnight and used as a starter culture for the following day's production. This method is still used for Grana cheeses: the whey at 50–55°C is incubated in Dewar flasks which maintain the high

temperature and select thermophilic microorganisms and consequently yield cultures of high purity.
2. *Undefined mixed-strain cultures*: these are selected by culture producers for their superior cheesemaking properties; they contain an unknown number of uncharacterised strains of LAB. These were the usual type of mesophilic culture used until recently and supplied by several culture suppliers.
3. *Defined-strain cultures*: these cultures were introduced in New Zealand in the 1930s, mainly to reduce the incidence of phage infection; they consist of one or more phage-unrelated strains of LAB which have been selected and blended based on their ability to produce high-quality cheese consistently. Many of these cultures are now produced as cell concentrates by culture suppliers and supplied in frozen form for direct addition to the milk in the cheese vat (direct vat starters, DVS). DVS cultures are more expensive than normal bulk cultures but since they are consistent and relieve the cheese company of the need for special laboratory facilities, DVS cultures are being used increasingly in large cheese factories.

Direct acidification using acid (usually lactic acid or HCl) or acidogen (GDL) is an alternative to biological acidification and is used commercially to a significant extent in the manufacture of Cottage, Quark, Feta-type cheese from ultrafiltration (UF)-concentrated milk and Mozzarella (Fox, 1978; Farkye, 2004). Direct acidification is more controllable than biological acidification and, unlike starters, is not susceptible to bacteriophage infection. However, enzymes from starter bacteria are essential in cheese ripening and hence chemical acidification is used mainly for cheese varieties for which texture and functionality are more important than flavour.

The rate of acidification depends on the amount and type of starter added and on the temperature profile of the curd and ranges from 5–6 hours for Cheddar and Cottage cheese, 10–12 hours for Dutch and Swiss types, to several days for cheese acidified by the endogenous microflora. The ultimate pH value of the curd for most rennet-coagulated cheeses is 5.0–5.3 but the pH value of acid-coagulated varieties (e.g. Cottage, Quark and Cream cheese) and some soft rennet-coagulated varieties (e.g. Camembert and Brie) is about 4.6.

If the cheese curds are cooked to below 40°C, a culture of *Lactococcus lactis* and/or *L. cremoris* is used but for high-cooked cheese (45–55°C), a culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* or *Lb. helveticus*, possibly in combination with *Streptococcus thermophilus*, is used. Cheese starters have been refined progressively over the years, especially with respect to the rate of acidification, resistance to bacteriophage and cheese-ripening characteristics. Today, mixtures of highly selected defined strains of LAB are used widely.

The production of acid at the appropriate rate and time affects several aspects of cheese manufacture and is critical for the production of good-quality cheese:

- Acidification affects coagulant activity during coagulation.
- Denaturation of the coagulant in the curd is reduced and its retention in the curd is increased as the pH is reduced, which influences the rate of proteolysis during ripening and may affect cheese quality.
- Acidification affects gel strength (curd tension), which influences cheese yield.
- Acidification affects gel syneresis, which controls the moisture content of cheese curd and hence regulates the growth of bacteria and the activity of enzymes in the cheese. Consequently, it strongly influences the rate and pattern of ripening and the quality of cheese.
- CCP in the casein micelles dissolves as the pH decreases, as a result of which the susceptibility of the caseins to proteolysis during ripening is increased and the rheological properties, meltability and stretchability of the cheese are modified.
- Acidification controls the growth of many undesirable bacteria in cheese, including pathogenic, food poisoning and gas-producing microorganisms; properly made cheese is a very safe product from the public health viewpoint.
- Acidification affects the activity of various enzymes in the cheese during ripening.

Some cheese varieties, for example Cheddar, are salted by mixing dry salt with chips of curd at the end of manufacture; since the level of salt in the cheese moisture rapidly reaches a value (5–6%) which halts the growth of LAB, the pH of curds for these varieties at salting must approximate the ultimate value (~5.2). However, most varieties are salted by immersing the formed cheese in brine or by surface application of dry salt; the diffusion of NaCl into the interior of the cheese is relatively slow and therefore there is ample time for the pH to decrease before the concentration of salt becomes inhibitory throughout the cheese. The pH of the curd for most cheese varieties is 6.2–6.5 at moulding and pressing but decreases to 5.0–5.2 during or shortly after pressing and before salting (see section 17.4.8).

In a few cases, for example Domiati, a high level of NaCl is added to the milk, traditionally to control the growth of adventitious microorganisms; this NaCl has a major influence on acid development, rennet coagulation, gel strength and curd syneresis.

17.3.2 Secondary cultures

The starter LAB dominate the microflora of cheese initially, but after reaching a maximum of about 10^9/g, they die off and lyse, and are replaced by a secondary microflora that comprises the following organisms:

1. Non-starter lactic acid bacteria (NSLAB), mainly adventitious mesophilic *Lactobacillus* spp., are normal contaminants from the milk, equipment or environment, and hence are variable in type and number. Depending on the temperature and other factors, the NSLAB reach 10^7–10^8 cfu/g relatively quickly (~1 month) and remain at approximately that number throughout ripening. After reaching their maximum number, the NSLAB remain viable but it is not known if some cells die and are replaced by new cells of the same or similar species. Because NSLAB dominate the viable microflora of long-ripened cheese and because of their variability, they are responsible for most of the variability of cheese quality; therefore, it is becoming increasingly common for Cheddar cheese to add selected strains of *Lactobacillus* as a component of the starter and which will outcompete the adventitious NSLAB.
2. Cultures of non-LAB are added to perform specific functions. These include *Propionibacterium shermanii* in Swiss-type cheeses, *Brevibacterium linens* in surface smear-ripened varieties, *Penicillium roqueforti* in blue cheeses, and *P. camemberti* and *Geotrichum candidum* in surface mould-ripened varieties. These are very active microorganisms and dominate the ripening of cheese in which they are used. They may be adventitious (from milk and environment) or added as a culture to the milk or curd (becoming increasingly common).

17.3.3 Coagulation

The essential step in the manufacture of all cheese varieties involves coagulation of the casein (and for a few varieties, the whey proteins also) of milk to form a gel which entraps the fat, if present. The coagulation of milk for cheese is achieved by one of three methods:

1. Rennet-induced coagulation, which is used for most ripened cheeses, and accounts for about 75% of total cheese production.
2. Acidification to about pH 4.6 at 30–36°C by *in situ* production of acid by fermentation of lactose to lactic acid by LAB (*Lactococcus*, *Lactobacillus* or *Streptococcus*) or direct acidification with acid or acidogen, usually GDL. Most acid-coagulated cheeses are consumed fresh and represent about 25% of total cheese production. Major examples of acid-coagulated cheeses are Cottage, Quark and Cream cheeses. Acid-coagulated cheeses are at one end of the spectrum of fermented dairy products, the production of which is summarised in Fig. 17.1a. Depending on the desired fat content of the final product, the starting material may be low-fat cream, whole milk, semi-skimmed milk or skimmed milk. The milk for Cottage-type cheese or Quark is subjected to a low heat treatment so that the syneretic properties of the coagulum are not impaired. These are consumed fresh as components of salads or as ingredients, for example in cheesecakes.
3. Acidification of milk, whey or mixtures thereof to about pH 5.2 and heating to about 90°C. The acid/heat coagulated cheeses are relatively minor varieties which are usually produced from rennet cheese whey or a blend of whey and skimmed milk and evolved as a means for recovering the nutritionally valuable whey proteins; they are usually consumed fresh, for example as hors d'oeuvres, or used as food ingredients. Important varieties are Ricotta (Italy), Anari (Cyprus) and Manori (Greece). If blends of milk and whey are used, it is necessary to adjust the pH of the blend to about 5.2 using vinegar, citrus juice or fermented milk. Acid/heat-coagulated cheese may also be produced from whole milk by acidifying to pH 5.2 and heating to 90°C, for example US-style Queso Blanco, which does not melt on heating and hence has interesting functional properties for certain applications.

A fourth, minor, group of cheeses is produced, not by coagulation, but by thermal evaporation of water from a mixture of whey and skimmed milk, whole milk or cream and crystallisation of lactose, for example Mysost and Gjetost; these cheeses, which are almost exclusive to Norway, bear little resemblance to rennet- or acid-coagulated cheese but resemble fudge.

17.3.4 Rennet-coagulated cheeses

These cheeses, which represent about 75% of total production, are produced in a great diversity of shapes, flavours and textures (at least 1000 varieties worldwide). Their production can be divided into two phases: (i) conversion of milk to cheese curd and (ii) ripening (maturation) of the curd to produce the final cheese (Fig. 17.1b).

The coagulation of milk for the production of rennet-coagulated cheese exploits a unique characteristic of the casein system. There are two principal groups of proteins in milk, caseins and whey proteins, in the approximate ratio of 4 : 1 for the four species of interest for cheesemaking; both groups of proteins are very heterogeneous. The four species of interest secrete four caseins, α_{s1}-, α_{s2}-, β- and κ-, but in different proportions (Table 17.1). The principal whey proteins are β-lactoglobulin (~50% of whey

Table 17.1. Proportions of caseins in milk of the principal dairying species.

Casein*	Bovine	Buffalo†	Caprine‡	Ovine
Total casein (%, w/w)	2.4–2.9	3.1–3.2	2.3–4.6	4.7–6.6
Individual caseins (% of total)				
α_{s1}	40	25–31	0–28	8
α_{s2}	10	12–16	1–25	12
β	35	21–34	0–64	60
κ	12	11–15	15–29	20
γ	3	0.15–0.16		

*The caseins are highly homologous across the four species.

†Based on data from Abd El-Salem & El-Shabiny (2011).

‡The wide variations in the proportions of caseins in caprine milk are due to complex genetic variability.

proteins), α-lactalbumin (~20%), blood serum albumin (~10%) and immunoglobulins (~10%) and perhaps 100 minor proteins at trace levels, including about 70 indigenous enzymes and proteins of the milk fat globule membrane. The whey proteins are not of direct consequence in cheese production but are denatured on heating and interact with the caseins with undesirable effects on the rennetability of milk; also, they are now widely recovered from whey and are valuable by-products of the cheese industry (Fox & McSweeney, 2003; McSweeney & Fox, 2013).

The α_{s1}-, α_{s2}- and β-caseins are precipitated by calcium at concentrations above 6 mmol/L. Since bovine, buffalo, ovine and caprine milks contain about 30, 45, 35 and 50 mmol/L of calcium, respectively, it would be expected that these proteins would precipitate in milk; however, they are prevented from doing so by the formation of large (diameter 50–600 nm, mean 150 nm) colloidal aggregates, known as casein micelles, which are stabilised by calcium-insensitive κ-casein, which is concentrated on the surface, with its hydrophobic N-terminal segment interacting with the α_{s1}-, α_{s2}- and β-caseins and its hydrophilic C-terminal third protruding into the aqueous environment, forming a layer about 7 nm thick, which stabilises the micelles by a zeta potential of about –20 mV and by steric stabilisation. The stability of the micelles is lost when the surface κ-casein layer is destroyed by heat, alcohol or proteinases (rennets). The structure and properties of the casein micelles has been studied in considerable detail since 1958 (i.e. since the discovery of κ-casein in 1956) and several models have been proposed but a unanimous view is lacking (Fox & McSweeney, 2003; Fox & Brodkorb, 2008; McSweeney & Fox, 2013).

Several proteinases can coagulate milk but the traditional, and the most effective, rennets were NaCl extracts of the stomachs of young, milk-fed calves, kids or lambs. The active enzyme in these rennets is chymosin (EC 3.4.23.4); as the animal ages, the secretion of chymosin decreases and is replaced by pepsin. Chymosin is an acid proteinase, i.e. a proteinase with two aspartic acid residues at the active site (residues 32 and 215 in the case of calf chymosin); calf chymosin has been crystallised and well characterised (Crabbe, 2004). Compared with other proteinases, chymosin is weakly proteolytic, with an optimum pH for general proteolysis at about 4.6 and is highly specific for hydrophobic residues. It seems to have evolved specifically for the coagulation of milk in the neonatal stomach where it is soon replaced by the more proteolytic pepsin.

The supply of chymosin has been inadequate for about 50 years due to the increased production of cheese and reduced availability of calf stomachs (due to the birth of fewer calves and the slaughter of calves at an older age). This shortage has led to a search for alternative coagulants (rennet substitutes). Many proteinases can coagulate milk under certain conditions but almost all are unsuitable as rennets because they are too proteolytic, resulting in a reduced yield of cheese curd and off-flavoured cheese. Only five successful rennet substitutes have been identified: bovine and porcine pepsins and acid proteinases from *Rhizomucor mehei*, *R. pusillus* and *Cryphonectria parasitica*. The calf chymosin gene has been cloned in *Kluyveromyces lactis*, *Escherichia coli* and *Aspergillus niger* and fermentation-produced chymosin is now used widely. The gene for camel chymosin has also been cloned in *A. niger* var. *awamori*. It has better clotting activity on camel milk than calf chymosin but is less proteolytic on bovine casein and therefore gives a slightly higher yield of cheese curd than calf chymosin (Bansal *et al*., 2009). Recent advances in milk clotting enzymes has been reviewed by Jacob *et al*. (2011).

Chymosin and most of the other commercially successful rennets hydrolyse κ-casein specifically at the Phe_{105}–Met_{106} bond but *C. parasitica* proteinase cleaves κ-casein at Ser_{104}–Phe_{105}. The Phe–Met bond is not inherently sensitive to chymosin; its sensitivity is determined by the sequence 98–111 of κ-casein, especially Ser_{104}. This sequence is highly conserved in the κ-casein from many species. Bovine, buffalo, ovine and caprine κ-casein contain a Phe–Met bond but the sectile bond in human, porcine, rat and mouse κ-casein is Phe–Ile (or Phe–Leu) which is readily hydrolysed by calf chymosin.

Cleavage of the Phe–Met bond releases the hydrophilic C-terminal segment, known as the (glyco) caseinomacropeptide (CMP), which diffuses into the surrounding aqueous

phase and the stability of the micelles is destroyed. When about 85% of the κ-casein has been hydrolysed, the rennet-altered micelles aggregate to form a gel in the presence of a critical concentration of Ca^{2+} and at a temperature greater than about 18°C; this is called the secondary (non-enzymatic) phase of rennet-induced coagulation (Horne & Banks, 2004). The precise reactions responsible for the aggregation of rennet-altered micelles are not known but involve hydrophobic interactions and calcium bridging; the gel can be dissolved by urea and calcium-sequestering agents, for example citrate. The rennet-altered micelles form into short chains initially and later into a three-dimensional gel (Fig. 17.2, see also Plate 17.1).

The development of the gel is usually monitored by rheological measurements, especially using a dynamic rheometer. Examples of the changes in the G′, G″ and tan δ are shown in Fig. 17.3. Aggregation may be monitored by electron microscopy or light scattering (Bansal *et al.*, 2007).

17.4 POST-COAGULATION OPERATIONS

17.4.1 Cutting the gel

After the gel has attained a certain firmness, which, traditionally, was determined subjectively by the cheesemaker, but more recently objectively using rheological measurements or light scattering techniques, it is subjected to a range of treatments, the primary objective of which is to remove moisture (whey) and concentrate the fat and casein in the form of cheese curds. For most varieties, the gel is cut/broken using implements which in some cases are traditional and characteristic of the variety. For most varieties, for example Cheddar, Gouda and Cottage, which are processed in rectangular vats, the coagulum is cut into cubes of about 1 cm, using vertical and horizontal knives; for traditional Swiss and Italian varieties, a 'harp' or 'spino', which are compatible with the hemispherical or conical vats, is used. In large modern vats, cutting knives are fixed in the vats and serve to cut the coagulum and stir the curds/whey (Kosikowski & Mistry, 1997; Fox *et al.*, 2000; Bennett & Johnston, 2004).

The optimum modulus for the gel at cutting is about 40 Pa, but varies from 20 to 60 Pa, depending on the protein content of the milk; losses of fat and protein in the whey increase with weaker or stronger gels. Rennet- or acid-coagulated milk gels are quite stable under quiescent conditions but if cut or broken, they synerese extensively, expelling whey.

17.4.2 Cooking the curds

After the gel has been cut and some whey expressed, the curds–whey mixture is cooked to a temperature and at a rate characteristic of the variety. The principal objective of cooking is to promote syneresis and thereby determine the moisture content of the cheese. Characteristic cook temperatures are Camembert 30°C (in effect no increase in temperature), Gouda about 35°C, Cheddar about 40°C, and Emmental and Grana cheeses about 55°C. The starter must be appropriate for the cook temperature: a mesophilic *Lactococcus* sp. for cheeses cooked at a temperature below 40°C and a thermophilic *Lactobacillus* sp. with or without *Streptococcus thermophilus* for a cheese cooked at a temperature above 40°C.

17.4.3 Syneresis

Syneresis concentrates the fat and casein of milk by a factor of 6–12, depending on the variety. The composition of the finished cheese is determined mainly by the extent of syneresis which initiates the differentiation of cheese varieties, although the type and composition of the milk, the amount and type of starter and the amount and type of rennet are also significant in this regard.

By controlling the rate and extent of syneresis, the cheesemaker controls the moisture content of cheese and thereby the rate and pattern of ripening and the quality and stability of the cheese. Syneresis is affected, *inter alia*, by concentrations of fat, protein and calcium, pH, size of curd particles, temperature of cooking, stirring of the curds–whey mixture and the curds after whey drainage, pressing of the curds, salting (2 kg H_2O lost per kg NaCl taken up).

When the desired degree of syneresis has occurred, as judged subjectively by the cheesemaker, the curds are separated from the whey, usually on some form of perforated metal screen. The curds are subjected to various treatments which are more or less variety specific. These include cheddaring, kneading–stretching, moulding, pressing and salting.

For small, high-moisture cheeses, the gel is scooped from the vat and transferred to perforated moulds, of variable size, where syneresis occurs; the cheese may be inverted in the moulds but is subjected to no other treatment.

The liquid expressed from the curds in the vat or in the moulds is called whey, which contains about 50% of the solids in milk (98% of the lactose, 25% of the protein and 10% of the fat). Until recently, whey was regarded as essentially worthless, to be disposed of as cheaply as possible. However, whey is now the source of valuable food products.

It is difficult/impossible to reproduce in-vat conditions on a laboratory scale and hence syneresis is the least-understood operation in cheesemaking at the molecular level. Principles that have been used to measure syneresis include: (i) measurement of free whey; (ii) weight of drained curd; (iii) electrical conductivity of the curd (as the moisture content of the curd decreases and the fat content

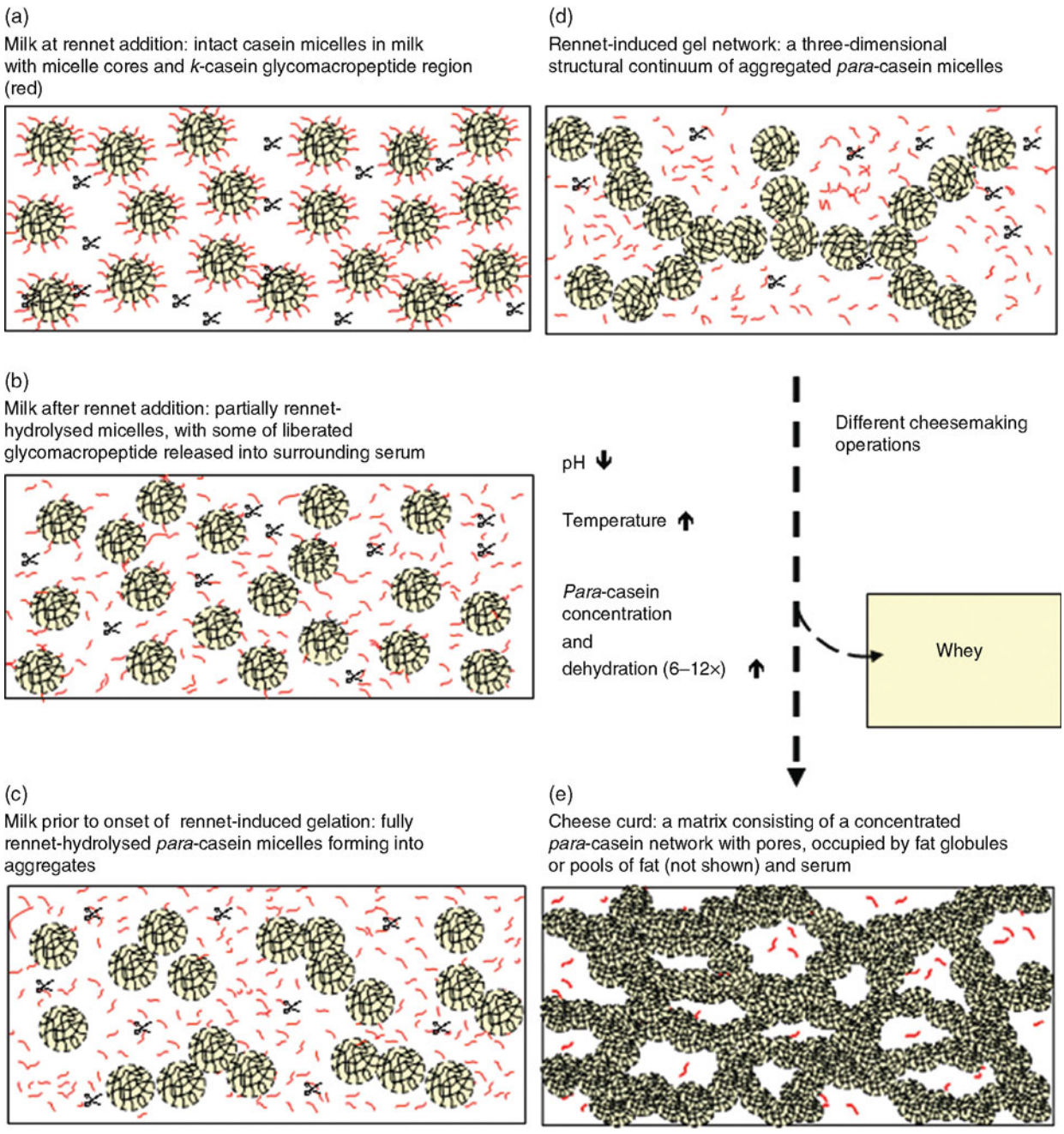


Figure 17.2. Schematic representation of the various stages involved in the formation of cheese curd from milk, starting from the initial mixture of casein micelles and added enzyme (rennet ✂) in the milk (a), and proceeding through rennet-induced hydrolysis of κ-casein (b, c), aggregation of *para*-casein micelles (c) and formation of *para*-casein gel network (d), which is dehydrated and concentrated into cheese curd (e). For a colour version of this figure, see Plate 17.1.

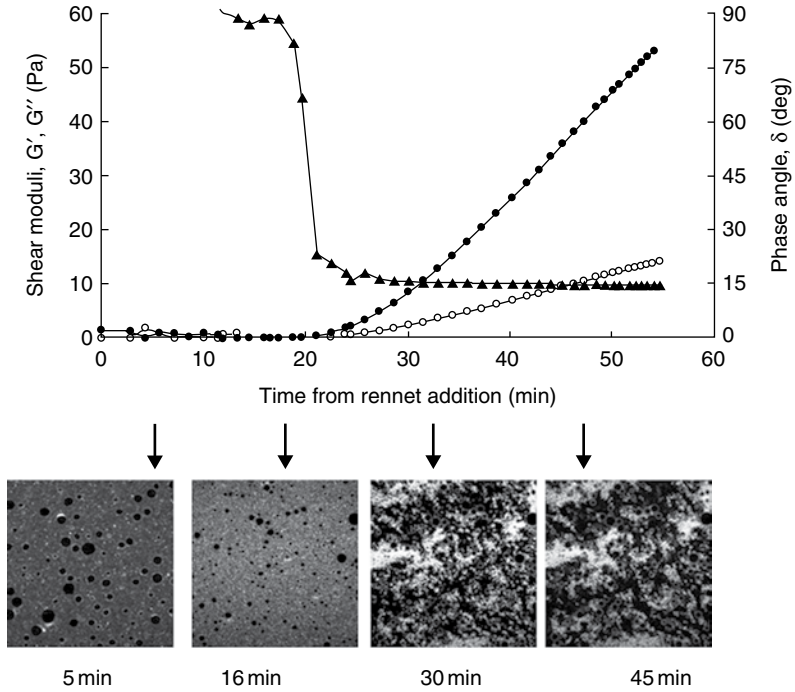


Figure 17.3. Changes in viscoelasticity and microstructure during rennet-induced gelation of milk, showing increases in elastic shear modulus (G′) and loss modulus (G″), a reduction in phase angle (δ), and aggregation of rennet-hydrolysed casein micelles into a network of *para*-casein micelles (as indicated by the increased localisation of white area in the confocal laser scanning micrographs).

increases, the electrical conductivity decreases); and (iv) layering a coloured or fluorescent dye or fat-free whey on the gel, so that as whey is expressed from the gel, the marker is diluted (the marker must not diffuse into or adsorb onto the gel). Syneresis is a first-order reaction which is affected, *inter alia*, by the size of the curd particles, temperature, pH and stirring of the curds–whey mixture (Dejmek & Walstra, 2004).

When the desired degree of syneresis has occurred, as determined subjectively by the cheesemaker, the curds are separated from the whey and are subjected to a series of operations, more or less unique for the manufacture of each cheese variety. Space does not permit a detailed description of the production protocols – the interested reader is referred to Fox *et al*. (2004) and several articles in Fuquay *et al*. (2011).

17.4.4 Draining the curd

For high-moisture cheeses, the curds and whey are transferred to perforated moulds where drainage occurs. For many varieties, for example Gouda, the cooked curds are allowed to settle in the vat and are pressed under the whey; the whey is drained off and the bed of curd cut into pieces of the required weight and placed in moulds for pressing. For Parmigiano Reggiano and traditional Emmental, the curds are scooped out of the vat using a heavy cloth and placed in moulds.

17.4.5 Cheddaring of the curd

For traditional Cheddar-type cheese, the curds were allowed to settle in the vat, the whey run off and the curds formed into banks, which are cut into pieces (~5 kg) and cheddared until the pH reached about 5.4 (MAFF, 1959). During cheddaring the pieces of curd flowed and developed a fibrous texture like chicken-breast meat, which was considered to be critical for development of the characteristic texture of Cheddar cheese. However, the principal physicochemical reaction during cheddaring is the decrease in pH, which dissolves the CCP and makes the curd stretchable. In modern Cheddar factories, the curds are drained on inclined moving perforated belts on which they are ‘cheddared’; a number of plants are available, for example the Alfamatic and Cheddar Master (Bennett & Johnston, 2004).

17.4.6 Curd washing

The curds for several varieties of cheese are 'washed' with water either before whey drainage (in the vat) or after whey drainage (on drainage tables, finishing vats or belts). Curd washing after whey drainage in traditional or farmhouse cheese manufacture may be done in the cheese vat once the whey is drained to the level of the curd bed. Washing before whey drainage involves removing a portion of the whey and diluting the remaining whey with warm (35–55°C) water while washing after whey drainage entails adding cold water (less than ~18°C) to the curd particles or curd chips and stirring the mixture. Cheeses that are washed in the vat before whey drainage are often referred to as Dutch-style cheeses (e.g. Gouda), while those varieties for which the drained curds are washed are referred to as washed-curd varieties, for example Colby (Davis, 1967; Kosikowski & Mistry, 1997).

The traditional manufacture of Gouda and Edam involves adding warm (50–60°C) water, equivalent to 20–25% of the volume of milk, to the coagulum after cutting to give a blend temperature of about 35°C. The original objective of this was to cook the curds in unjacketed vats but it also reduces the lactose content of the curds and thus avoids over-acidification and yields a milder, sweeter cheese. For milk containing about 4.8% lactose, increasing the wash water from zero (non-washed) to 0.33 kg/kg milk increases the pH of mature Cheddar-style cheese by about 0.4 units (Hou *et al*., 2012). Washing has little effect on the calcium content of curd because much of the calcium is colloidal at the pH (~6.15–6.35) and temperature (36–39°C) at whey drainage. Also, washing does not influence the level of proteolysis, as measured by levels of pH 4.6-soluble nitrogen and free amino acids. Hence, altering the level of curd washing provides a useful means of altering curd pH, which influences ripening, appearance, flavour and physical properties (e.g. sliceability, shreddability, elasticity). Varying the level of curd washing pro rata the level of lactose in the milk enables the manufacturer to make cheese that consistently reaches the target pH from a milk supply having seasonal variations in lactose level (Guinee & O'Callaghan, 2010). Standardisation of lactose, and lactic acid, in the moisture phase of cheese manufactured from milk obtained mainly from spring-calved cows, by curd washing, at a level proportional to the lactose content of the milk favours more consistent cheese in terms of pH and composition. The lactic acid content of Cheddar cheese, which is not washed in the vat, can vary dramatically (Guinee *et al*., 2008). In New Zealand and Ireland, milk production is very seasonal and its composition is variable; *inter alia*, the lactose content decreases from about 4.8% in early lactation to about 4.3% in late lactation. The variable lactose content of milk is reflected in the lactose and lactic acid content of the cheese and has a considerable effect on the quality of cheese.

Cheddar cheese curd produced from high (8.4%) lactose milk contained about 2.5% lactose which had been fermented during ripening but after 180 days of ripening, the cheese still contained 1.4% lactose; the pH of the cheese decreased to 4.7 during ripening and the cheese had a sharp, strong flavour; in contrast, if the lactose content of the milk was reduced, the cheese was free of lactose after 60 days of ripening and had a mild flavour (Shakeel-ur-Rehman *et al*., 2004). Washing also reduces the buffering capacity of the cheese by reducing the level of CCP and consequently the pH increases during ripening, to about 5.8 in mature Gouda.

The drained curds for two variants of Cheddar (Colby and washed-curd Cheddar) and Monterey cheese are washed by adding cold water (≤18°C) to the drained curd, at a level of about 25% of the milk volume, when the whey has been drained to the top of the curd bed. The curd–whey wash water mixture is stirred for 5–15 min and the curds are then drained, salted, moulded and pressed. In the modern commercial manufacture of Colby using CheddarMaster or Alfamatic systems, the curds are washed by spraying cold water onto the bed of curds on the rotating perforated drainage belts which are agitated continuously by overhead-mounted peg stirrers to prevent the curd particles knitting and to ensure good contact between wash water and curd particles.

The primary function of washing curd after whey drainage is to cool the curds, reduce curd knitting and syneresis, and thereby increase cheese moisture, reduce hardness, and give milder-flavoured cheese. Washed-curd cheeses appear to be whiter than their unwashed equivalents, due to a lower degree of casein aggregation

The curds for Cottage cheese and *pasta filata* cheeses, such as Mozzarella and Kaschkaval, are also washed but these cheeses are not considered as washed-curd varieties.

In the manufacture of Cottage cheese, chlorinated, acidified (pH ~5.5–6.0) water at about 25, 10 and 0°C is added sequentially in appropriate quantities to reduce the temperature of the curds from about 55 to below 10°C (Farkye, 2004). The functions of curd washing are to ensure Cottage cheese with the desired granular consistency, by reducing the temperature of the curd particles and thereby their tendency to clump; reducing the lactate content and thereby maintaining the pH at 4.8–4.9; minimising the loss of calcium attached to aspartate and glutamate residues and avoiding a soft 'mushy' texture and giving the desired mild taste and shelf-life (about 2–3 weeks).

In the manufacture of *pasta filata* cheeses, the treatment (kneading and mixing) of curd with added hot water

(~70–80°C) was used originally to 'pasteurise' the curd and improve its microbiological status and keeping quality. The kneading/mixing of the curds in hot water impart desired textural (chewiness) and cooking (stringiness, with desired degrees of oiling-off, succulence, gloss) characteristics to the cheese. The development of a range *pasta filata* cheeses, for example Mozzarella, Kachkaval and Halloumi, with unique characteristics evolved over time (Kosikowski & Mistry, 1997; Kindstedt *et al*., 2004). The functions of washing in modern manufacture are to ensure the desired texture and end-product characteristics. Hot water treatment of the curd at the desired pH (~5.2–5.4) raises the temperature to about 58–60°C and promotes a number of structural changes that impart the characteristic fibrous, stringy, 'oily', glistening texture of the cooked cheese by aggregation of the *para*-casein matrix into fibres of high tensile strength; coalescence of fat globules into pools of free fat, which are entrapped between the casein fibres; and inactivation of residual coagulant in the curd, which reduces the level of proteolysis in the cheese during storage, and thereby minimises deterioration of the stringiness.

17.4.7 Moulding and pressing

Cheeses are made, traditionally, into characteristic shapes and size, for example Camembert, small low cylinders (~200 g); Limburger and Telligo, shallow squares; Blue, cylinders (~5 kg); Edam, spheres (~5 kg); Cheddar and Parmigiano Reggiano, tall cylinders (~20 and 45 kg, respectively); Emmental and Gruyère, large low cylinders (60–80 kg); Provolona, pear shaped. Presumably, the traditional shapes reflected the moulds available when the particular cheese evolved and to a large extent are cosmetic. Many of the traditional shapes persist but some have been replaced, for example Cheddar is now usually made in 20-kg rectangular blocks. However, the size of a cheese is not only cosmetic: surface-ripened cheeses must be small because ripening is dominated by the surface microflora and the enzymes and products secreted by them, which diffuse to only small distances into the cheese. At the other extreme are cheeses with a propionic acid fermentation which must have a close texture and be large enough to retain sufficient CO_2 for eye development. In an 80-kg Emmental cheese, 120 L of CO_2 are produced during maturation, 60 L of which remain dissolved in the cheese body, about 20 L is in the eyes and about 40 L diffuses out of the cheese (Frohlich-Wyder & Bachmann, 2004). If the cheese has an open texture, too much CO_2 will be lost and too little retained to form eyes. Traditional cheese moulds were made of wood or tinned steel but today, stainless steel or plastic are used.

Curds for high-moisture cheeses form a congealed mass under their own weight, but the curds for medium- and especially for low-moisture cheeses must be pressed to give a well-matted body; the lower the moisture content of the curds, the higher the pressure that may, and must, be applied (e.g. Cheddar cheese is pressed at 2.7 kPa). In addition to consolidating the mass of curds, pressing removes some whey (for Cheddar cheese, ~1.3% of the total volume of milk used).

17.4.8 Salting

Most, probably all, cheeses are salted at the end of curd manufacture (Fox *et al*., 2004). Salt, which varies from about 2 to 10% in the moisture phase, has a major influence on various aspects of cheese ripening, quality and safety:

1. Salt has a direct effect on flavour.
2. Salt reduces the water activity, a_w, of cheese; the a_w of very young cheese is determined almost entirely by its NaCl content: $a_w = 1 - 0.033\ [NaCl_m] = 1 - 0.00565\ [NaCl]$, where $[NaCl_m]$ is the molality of NaCl and [NaCl] is the concentration of NaCl as g/100 g cheese moisture. As the cheese ages, some compounds produced during ripening, for example amino acids, small peptides and fatty acids, also influence a_w. Reduction in water activity of Cheddar cheese (from about 0.97 to 0.96) during maturation is strongly correlated with increases in the concentrations of water-soluble peptides and free amino acids and with starter culture autolysis. The a_w of selected cheese varieties is shown in Table 17.2.

Table 17.2. Variation in water activity (a_w) of selected cheese varieties.

a_w	Cheese
1.00	Fresh cheese curd, Ricotta
0.99	Beaumont, Cottage, Quark
0.98	Belle des Champs, Münster, Pyrénées, some processed cheeses, Taleggio
0.97	Brie, Camembert, Emmental, Fontina, Limburger, Saint Paulin, Serra da Estréla
0.96	Appenzeller, Chaumes, Edam, Fontal, Havarti, Mimolette, Norvegia, Samsø, Tilsit
0.95	Bleu de Presse, Cheddar, Gorgonzola, Gouda, Gruyère, Manchego
0.94	Idiazábal, Majorero, Mozzarella, Norzola, Raclette, Romano, Sbrinz, Stilton
0.93	Danablu, Edelpilzkäse, Normanna, Torta del Casar
0.92	Castellano, Parmesan, Roncal, Zamorano
0.91	Provolone, Roquefort
0.90	Cabrales, Gamalost, Gudbransdalsost, Primost

Data compiled from various sources.

Table 17.3. Minimum water activity (a_w) required for the growth of selected microorganisms in foods.

Pathogen	Minimum a_w
Shigella spp.	0.96
Yersinia enterocolitica	0.96
Vibrio parahaemolyticus	0.94
Pseudomonas spp.	0.95
Escherichia coli	0.95
Clostridium botulinum	0.94
Salmonella spp.	0.94
Listeria monocytogenes	0.92
Micrococcus spp.	0.87
Staphylococcus aureus	0.86
Most yeasts and moulds	0.80
Osmophilic yeasts and moulds	0.55

Data compiled from various sources.

3. The growth and survival of bacteria is strongly affected by a_w (Table 17.3). Propionic acid bacteria are very sensitive to NaCl; on the contrary, the germination of *Penicillium* spores is stimulated by NaCl.
4. The activity of chymosin and microbial enzymes is affected by NaCl and may lead to abnormal ripening, for example bitterness.

Cheese is salted by one of three methods:

1. *Dry salting*: mixing dry salt with curd chips, for example Cheddar, Stilton and Cottage; the NaCl dissolves in moisture at the surface of the chips and diffuses into the curd; since the chips are quite small (~1 cm^2 cross-section), salt equilibrium is established within 24 hours throughout the chips. However, if the salt is not distributed uniformly on the chips initially, equilibrium will never be established. Salt uptake is affected by temperature and whey exudes from the curds in response to the increasing osmotic pressure in the curd; approximately 50% of the NaCl applied to the curds is lost in the whey, which contains 3–4% fat and 1–1.3% protein (mainly whey proteins).
2. *Brine salting*: submersion of moulded cheese in brine containing about 18–25% w/w NaCl and 0.2% w/w added calcium and adjusted to pH about 5.2, as practised for Gouda, Emmental, Grana and Camembert. The curds for brine-salted cheese varieties are formed into their final size and shape prior to immersion in brine for a period ranging from about 0.5 hours to 10 days, depending on the brining conditions (e.g. salt content, temperature) and cheese variety and size.
3. *Surface dry salting*: rubbing dry salt on the surface of pressed cheese (surface dry salting), for example blue cheese. In principle, salting by this method is similar to brine-salting, the main difference being that solid salt first forms a brine in moisture absorbed from the cheese before inward diffusion of NaCl.

17.4.8.1 Nutritional significance of salt in cheese

NaCl (specifically sodium) increases blood pressure and an excess is undesirable; the recommended daily allowance (RDA) (USA, UK) is about 2.4 g of sodium. The principal sources of sodium are processed foods (Kilcast & Angus, 2007). Although natural cheese makes a relatively small contribution to the intake of NaCl (consumption of 20 kg per annum of cheese containing 2% NaCl, which is at the upper end of consumption, contributes 400 g of NaCl per annum, i.e. about 1.1 g NaCl or 0.7 g of sodium daily), there is a commercial incentive to reduce the level of salt in cheese. Approaches by which this might be achieved include simply reducing the salt content of cheese – this could be done to only a limited extent, i.e. not less than 4.6% salt-in-moisture (S/M) in the case of Cheddar cheese, without the risk of flavour defects. Replacing NaCl with KCl or $MgCl_2$ causes bitterness but up to about 15% of the NaCl can be substituted by KCl without adverse effects; milk salts remaining after crystallisation of lactose from UF permeate and which also contains amino acids and other organic compounds has been recommended as a substitute for NaCl.

17.4.9 Packaging

The ripening of rennet curd cheeses can vary from a few weeks to 1–2 years. During this period, moisture evaporation from the surface of unwrapped cheese results in the development of a rind (up to 5 mm thick), or enhances the rind formed under brining conditions that promote dehydration and fat loss from the outer layer. The resultant rind, which is in effect a dehydrated layer of calcium phosphate *para*-casein with a low fat content, behaves as a strong natural 'package' which maintains the cheese shape, minimises moisture loss from the interior, protects against breakage and imparts a certain aesthetic to the cheese, for example when the rind acquires a distinctive patterned mould encrustation or is treated with olive oil and paprika. However, the rind is generally inedible, difficult to process (e.g. shredding, or in the manufacture of processed cheese), and is generally discarded. For small high-moisture, especially surface-ripened, cheeses, rind formation is minimised by storing the cheese in a high-humidity atmosphere and washing the surface.

Many ripened cheeses are now wrapped in various materials, the main functions being to give physical protection, reduce moisture loss, prevent the growth of spoilage and

undesirable microorganisms and, in some cases, to impart distinctiveness that serves to characterise the cheese. Traditionally, medium- and low-moisture rennet curd cheeses were coated with wax (black, red or yellow) but waxes have been replaced by polymer preparations, which are heated, applied by spraying and brushing onto the cheese surface, and form a tight layer around the cheese on cooling. An example of the latter is Plasticcoat®, a widely used yellow covering on Dutch-style cheeses, which consists of an emulsion of vinyl acetate, natamycin (Delvocid®, an antimycotic agent) and water-soluble food-grade colours. Multilayered polymer flexible heat-sealable films are widely used to wrap rindless, internally ripened hard cheese, such as Cheddar, Mozzarella, Jarlsberg and Emmental. Films are usually composed of a composite of polymers, each contributing to specific functions in terms of tensile strength, stretchability, shrinkability on application of vacuum and/or heat, permeability to gasses (CO_2, N_2, O_2) and moisture, heat sealability and clarity to permit visual quality checks: for example, polyamide, high-density polyethylene, ethylene-vinyl alcohol (EVOH), ethylene-α-olefin, polypropylene, polypropylene-ethylene, ethylene vinyl acetate (EVA). Examples of such films used for bulk packing of blocks and wheels include Coextruded Plastic Technologies, Inc. (CPT) films (e.g. Plastobarr LH, Plastofresh), and Cryovac® (e.g. multilayered Cryovac®, B2100), General films (VF/1 M), MandQ Packaging Corporation.

Films are designed to:

- maximise the permeation of CO_2 (which is produced by respiring microorganisms) out of the film-wrapped cheese, so as to avoid the development of holes, cracks, and/or the 'ballooning' of the film;
- minimise the transmission of O_2 into the cheese, to reduce the risk of surface mould growth;
- provide strength and stiffness, which on vacuuming contributes to consolidation of the warm cheese mass to the desired shape, restraining the cheese and thereby enhancing internal knitting throughout the cheese mass.

Soft cheeses and fresh acid curd cheeses such as Quark, Mascarpone, Cream cheese and Cottage cheese are relatively short shelf-life, high-moisture, soft products. Unlike most rennet curd cheeses, they are incapable of retaining their shape during wholesale and distribution. They are packed in various types of material, including lacquered aluminium foils, tubs (sometimes under a controlled atmosphere) made from paperboard, or composites of various polymers, including high impact polystyrene, polythene, polyethylene terephthalate and polyvinylidene chloride. The package serves to prevent contamination of the cheese from the environment, to prevent the loss of moisture and as a means of product identification and advertising. Polylactic acid films, derived from corn starch, a biogradable polymer, is a satisfactory alternative to hydrocarbon-based polymers for the packaging of soft cheese. Useful references on packaging include Newsome and Arnold (1986), Lulham and Toney (1997), Paleari and Barbaglia (2000) and Broda (2001).

17.5 MEMBRANE PROCESSING IN CHEESE TECHNOLOGY

Since cheesemaking is essentially a concentration process, it is not surprising that membrane-based processing has found many applications in the cheese industry (Renner & Abd El-Salam, 1991; Mistry & Maubois, 2004; Mistry, 2011). It is customary to subdivide membrane processing into four categories, based on the porosity (P) and molecular mass cut-off of the membranes:

1. *Reverse osmosis* (RO; hyperfiltration; P, 0.0001 μm; 100 Da) removes only water from milk or whey, and is an alternative to thermal evaporation. RO has little or no application in cheese technology.
2. *Nanofiltration* (NF; P, 0.001 μm; 500 Da) removes water and small monovalent ions (sodium, potassium, chlorine) as permeate and retains concentrated milk. NF has been used for on-farm concentration of milk to reduce transport cost but has little application in cheese technology.
3. *Ultrafiltration* (UF; P, 0.01–0.005 μm; 5–10 Da) separates water, lactose, soluble salts and vitamins in the permeate from proteins (caseins and WPs) in the retentate. UF is the most widely used membrane-based technique in the cheese industry.
4. *Microfiltration* (MF; P, 0.14 μm; 200 kDa) uses large-pore membranes to separate casein micelles in the retentate from the serum containing whey proteins, water and other small molecules. Using larger pore membranes (1.4 μm), casein micelles will permeate and only bacteria are retained. This process, known as the Bactocatch system, may be used as an alternative to bactofugation to treat milk for cheeses susceptible to 'late blowing' as a result of the growth and gas (CO_2 and H_2) production by *Cl. tyrobutyricum*, for example Gouda and Swiss varieties.

In all applications of membrane processing in the dairy industry, the fat globules are generally first removed by centrifugation and if necessary the cream and retentate (or in some cases the permeate from the Bactocatch process) are recombined. If not removed efficiently, the fat globules will block the membranes. In large, modern cheese factories, where UF and/or MF are used to standardise milk

protein or casein, several streams (raw milk, skimmed milk, retentate, permeate and cream) may be blended to give the desired composition.

Depending on the objectives, various degrees of concentration may be obtained:

1. *Low concentration factor UF (LCF-UF)* (1.0–1.5×, ≤4.5% protein) is now widely used to standardise, and increase, the casein concentration in cheese milk. It offers several advantages, especially when used in conjunction with in-vat curd firmness sensors and gel cutting based on firmness: it facilitates closer adherence to standard operating procedures, more consistent cheese composition and greater accuracy in cheese yield prediction, and increases the capacity of the cheese plant. Early studies reported that cheese made from LCF-UF milk underwent less primary and secondary proteolysis, was firmer, and matured more slowly than cheeses made from control milk. However, when the ratio of rennet dosage to casein load (mL rennet/kg casein) in LCF-UF milk was increased to that of the control cheese, the differences disappeared.
2. *Medium concentration factor UF (MCF-UF)* (2–5×) is used commercially for the manufacture of relatively high-moisture rennet curd (e.g. Camembert and Brie, some Blue varieties and cast Feta) and acid curd cheese varieties (Cream, Quark and Ricotta). Two basic approaches are used with MCF-UF:
   a. *Full concentration UF* to the final total solids content of the cheese, with gelling and acidification of the retentate to the final cheese, without whey expression.
   b. *Part concentration UF* of the milk, setting the retentate to form a curd from which whey is expressed by various means.
3. *Concentration by MF*. For most rennet curd cheeses, especially hard varieties (e.g. Cheddar, Swiss, Mozzarella), the inclusion of more than 1.5% w/w whey proteins in cheese can adversely affect quality, for example impair syneresis, resulting in higher moisture cheeses, and alter texture (e.g. cheese becomes softer, less elastic, less capable of retaining gas as eyes) and cooking properties (reduced meltability, stringiness and stretchability) (Guinee, 2003).

Full concentration involves concentrating the milk to the gross composition of the final cheese, and the retentate referred to as a liquid pre-cheese, is inoculated with starter culture (and/or GDL) and rennet. Then, it may be filled into the final package (mould) which is incubated at a controlled temperature to allow the rennet-treated retentate to set to the final product, referred to as a 'cast cheese', as it has a smooth uniform structure devoid of curd particle or granule junctions. Alternatively, the inoculated rententate may be set (gelled) and subsequently structured by cutting into cubes (usually on specialised equipment, e.g. Alcurd), moulding the cubes and pressing; a small amount of whey may be lost at this stage. Curd structuring in this way allows curds to be treated as in conventional manufacture, for example addition of blue mould spores and salt, and plasticisation.

The use of MCF-UF (5×) to partly concentrate the cheese milk was envisaged as a method for producing hard cheeses, for example Cheddar, by UF. The process involved UF to as high a concentration as possible (~18–20% protein), setting the retentate, cutting the resultant gel, and subjecting the curds to syneresis to increase the dry matter content to that required in the cheese. The post-gelation operations were carried out using specialised equipment (e.g. Sirocurd). It was claimed that the retention of whey proteins in the curd would increase cheese yield by 6–8%, but this was not achieved in practice and the retained whey proteins adversely affected the texture of the cheese and retarded proteolysis and flavour development; the process was abandoned.

17.6 RIPENING

Acid-coagulated cheeses are ready for consumption at the end of curd manufacture. Although rennet-coagulated cheese may be consumed as fresh curd, and a little is, for example Junket or Burgos cheese, most is ripened (matured) for a period ranging from about 2 weeks (Mozzarella) to over 2 years (Parmigiano-Reggiano, extra-mature Cheddar) during which the characteristic flavour, texture and functionality of the cheese develop. Generally, the duration of ripening is inversely related to the moisture content of the cheese. Many varieties may be consumed at any of several stages of maturity, depending on the flavour preferences of consumers and economic factors.

Although curds for different cheese varieties are recognisably different at the end of manufacture (due mainly to compositional and textural differences), the unique characteristics of each variety develop during ripening as a result of a complex sequence of biochemical reactions. The changes that occur during ripening, and hence the flavour, aroma and texture of the mature cheese, are predetermined by the manufacturing process, especially by the levels of moisture and NaCl and pH, residual coagulant activity, the type of starter and, in many cases, by the secondary microflora (added or adventitious).

During ripening, a very complex series of reactions, which fall into three groups, occurs:

1. glycolysis of lactose and modification and/or catabolism of the resulting lactate, and in some varieties of citrate also;

2. lipolysis and the modification and catabolism of the resulting fatty acids;
3. proteolysis and catabolism of the resulting amino acids.

17.6.1 Ripening agents

The biochemical changes which occur during ripening are caused by one or more of the following agents (Fox *et al*., 2004):

1. *Coagulant*: depending on the coagulant used, the pH of the curd at whey drainage, the temperature to which the curds are cooked and the moisture content of the curds, 0–30% of the rennet added to the milk is retained in the cheese curd.
2. *Indigenous milk enzymes*: these are particularly important in raw-milk cheese but many indigenous enzymes are sufficiently heat stable to withstand high-temperature short time (HTST) pasteurisation. Important indigenous enzymes include plasmin, xanthine oxidoreductase, acid phosphatase and, in raw-milk cheese, lipoprotein lipase.
3. *Starter LAB and their enzymes*: these reach maximum numbers (~10^9 cfu/g) at the end of curd manufacture (6–12 hours). They then die, or at least cannot be cultured, and lyse at strain-dependent rates, releasing their intracellular enzymes (Sheehan *et al*., 2005). Since all the enzymes of LAB, except the cell membrane-bound proteinase, are intracellular, they contribute to ripening only when the cells lyse, or at least become permeable. Probably the most important LAB enzymes are the peptidases, of which LAB possess a wide range.
4. *NSLAB*: these are adventitious LAB which contaminate the cheese milk at farm and/or factory. Traditionally, cheesemakers relied on NSLAB for acid production during curd manufacture; this is still the case with some minor artisanal varieties but starter LAB are now used in all factory and most artisanal cheesemaking. Most NSLAB are killed by HTST pasteurisation, and curd made from good-quality pasteurised milk in modern enclosed equipment has only a few hundred NSLAB, mainly mesophilic lactobacilli, per gram at the start of ripening. However, they grow at a rate depending mainly on the temperature to 10^7–10^8 cfu/g within about 3 months. In cheese made from raw milk, the NSLAB microflora is more diverse and reaches a higher number than in cheese made from pasteurised milk; the difference is probably mainly responsible for the more intense flavour of the former compared with the latter. This situation applies to all cheese varieties that have been investigated and the NSLAB dominate the viable microflora of cheese ripened for more than 2 months.
5. *Secondary cultures*: most cheese varieties develop a secondary microflora, which was originally adventitious but now develops mainly from added cultures. Examples are *Propionibacterium freudenrichii* subsp. *shermanii* (Swiss cheeses), *Penicillium roqueforti* (blue cheeses), *P. camamberti* and *Geotrichum candidum* (Camembert and Brie), *Brevibacterium linens*, *Arthrobacter* spp., *Corynebacterium* and yeasts (surface smear-ripened cheese), citrate-positive *Lc. lactis* and *Leuconostoc* spp. (Dutch-type cheeses). Most of the microorganisms used as secondary cultures are very active metabolically and secrete very active proteolytic and lipolytic enzymes. Consequently, they dominate the ripening of varieties in which they are used. Traditionally, a secondary culture was not used for Cheddar-type cheese but it is becoming increasingly common to add an adjunct culture, usually *Streptococcus thermophilus* and mesophilic or thermophilic lactobacilli, to accelerate ripening, intensify flavour and perhaps tailor-make flavour; essentially, the objective is to reproduce the microflora and flavour of raw-milk cheese.
6. *Exogenous enzymes*: for some varieties of Italian cheese, for example Provolone and Pecorino varieties, rennet paste which contains a lipase, pregastric esterase (PGE), in addition to chymosin, is used as coagulant; PGE is responsible for extensive lipolysis and the characteristic piquant flavour of the cheese.

17.6.2 Ripening reactions

17.6.2.1 Glycolysis and related events

Fresh cheese curd contains about 1% lactose, which is converted to lactic acid (mainly the L-isomer) by the starter LAB, usually within 24 hours. Depending on the variety, the L-lactic acid is racemised to DL-lactic acid by NSLAB, or catabolised to CO_2 and H_2O in mould-ripened and smear-ripened cheese (Fig. 17.4). In Swiss-type cheese, lactic acid is converted to propionic and acetic acids, CO_2 and H_2O by *P. freudenrichii* subsp. *shermanii*; the CO_2 is responsible for the characteristic eyes in such cheese.

The pH of most cheeses increases during ripening due to the catabolism of lactic acid and in the case of smear- or mould-ripened varieties to the production of NH_3 at the surface, which diffuses into the cheese; the pH of Camembert increases from about 4.6 to about 7.5. The pH of Cheddar does not normally increase, probably because the catabolism of residual lactose (~1%) in the curd reduces the pH to a value below the buffering maximum of cheese (pH ~5.2). If the lactose content of milk for Cheddar cheese is reduced or if the curd is washed, the post-manufacture decrease in pH is avoided and the pH increases in a similar way to Gouda; washed-curd or reduced-lactose Cheddar has a mild flavour while high-lactose Cheddar has a harsh, strong flavour (Shakeel-ur-Rehman *et al*., 2004).

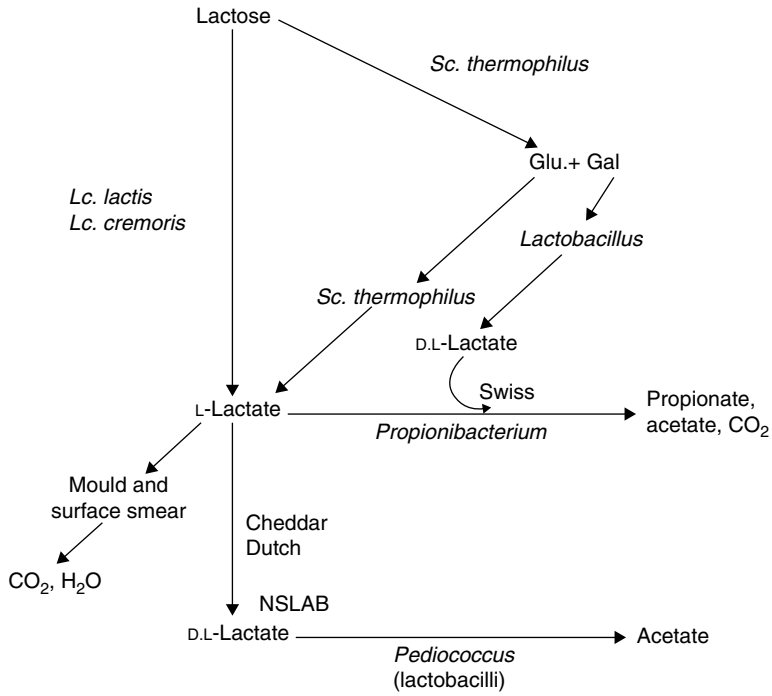


Figure 17.4. Summary of lactose metabolism in cheese.

17.6.2.2 Lipolysis

Little lipolysis occurs in most cheese varieties, in which it is catalysed by the weakly lipolytic LAB or NSLAB, and indigenous milk lipase if raw milk is used. Extensive lipolysis occurs in some hard Italian-type cheeses, for example Provolone and Pecorino varieties, for which pre-gastric esterase is responsible, and in Blue cheese, for which *P. roqueforti* is responsible. Fatty acids are major contributors to the flavour of Provolone and Pecorino cheese and some may be converted to lactones or esters, which have characteristic flavours. In blue-veined cheese, fatty acids are converted to methyl ketones by *P. roqueforti* and these are mainly responsible for the characteristic peppery taste of such cheese (Fig. 17.5). Some of the methyl ketones may be reduced to secondary alcohols, which cause off-flavours.

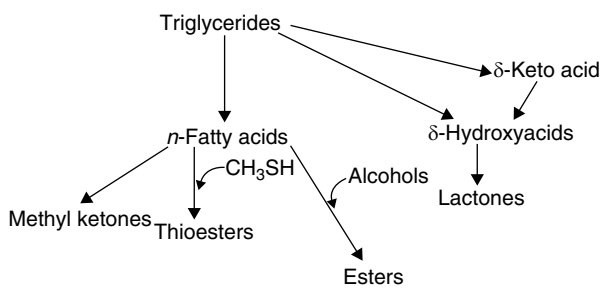


Figure 17.5. Summary of lipolysis in cheese.

17.6.2.3 Proteolysis

Proteolysis is the most complex and probably the most important of the three primary ripening reactions for the quality of cheese, especially internal bacterially ripened varieties. Initially, the caseins are hydrolysed by chymosin, and to a lesser extent by plasmin, in the case of β-casein; chymosin is almost completely inactivated in high-cook varieties and in this case plasmin is the principal agent of primary proteolysis. The polypeptides produced by chymosin and plasmin are too large to affect flavour, but primary proteolysis has a major influence on the texture and functionality of cheese. The peptides produced by chymosin and plasmin are hydrolysed to smaller peptides and amino acids by proteinases and peptidases of starter LAB and NSLAB. Small peptides contribute positively to the background brothy flavour of cheese but some are bitter. Many amino acids also have a characteristic flavour but, more importantly, they serve as substrates for a great diversity of catabolic reactions, including decaboxylation, deamination and transamination (Fig. 17.6) catalysed by

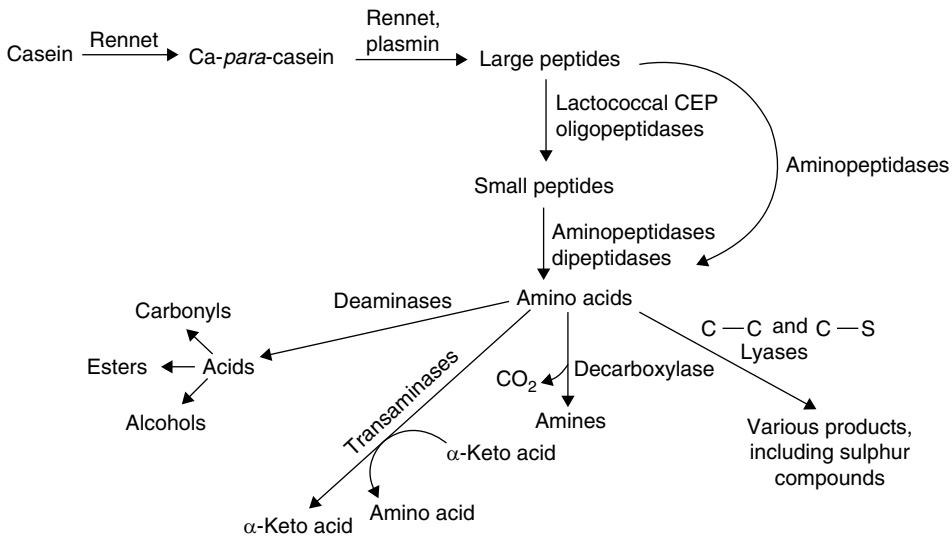


Figure 17.6. Summary of proteolysis and amino acid catabolism in cheese. CEP, cell envelope proteinase.

enzymes of LAB, NSLAB and the secondary culture. The flavour of internal bacterially ripened cheese is probably due mainly to compounds produced from amino acids.

Owing to its complexity, a wide range of analytical techniques are available to assess the extent and depth of proteolysis, including the following (Ardo & Polychroniadou, 1997): polyacrylamide gel electrophoresis (sodium dodecyl sulphate or urea) of whole cheese or fractions thereof; reverse phase high-performance liquid chromatography (RP-HPLC); formation of water-soluble, pH 4.6-soluble, ethanol- or trichloroacetic acid-soluble nitrogen; liberation of amino groups; analysis of amino acids by ion exchange chromatography or RP-HPLC.

17.6.3 Accelerated ripening of cheese

Cheese ripening is a slow process, ranging from about 2 weeks for Pizza cheese to more than 2 years for Parmigiano Reggiano and extra-mature Cheddar. Ripening is expensive owing to the cost of inventory, the need for controlled-temperature ripening rooms and the risk of defects. Consequently, there are economic and technological incentives for accelerating the cheese-ripening process provided that the flavour and texture of the cheese do not deteriorate. Proteolysis is probably the limiting reaction in the ripening of most varieties and most methods used to accelerate ripening are aimed primarily on proteolysis. These fall into one of the following categories: elevated ripening temperature, addition of exogenous enzymes, addition of chemically or physically modified bacterial cells to the cheese, use of genetically modified starters, use of adjunct cultures, use of cheese slurries or enzyme-modified cheese.

Each of these methods has advantages and disadvantages as summarised in Table 17.4 (Wilkinson, 1993; Fox *et al*., 1996; Kilcawley *et al*., 1998).

17.7 FACTORS THAT AFFECT THE QUALITY OF CHEESE

The quality of cheese involves at least the following attributes (Fox & Cogan, 2004): appearance, flavour, texture, functionality, microbiological safety and nutritional value.

The appearance of cheese is usually the only attribute which the consumer can assess when purchasing cheese and hence is critically important. Appearance includes colour, the presence or absence, as appropriate for the variety, of mould and the presence or absence, as appropriate, of eyes or other openings. From experience, the consumer knows that if the appearance of cheese is defective, so will be its flavour, texture and functionality. Today, the appearance of cheese presented by retailers is normally acceptable – defective batches will have been rejected during the selection process.

The consumer cannot assess microbiological quality and relies on the manufacturer, retailer or public health inspectors to ensure the microbial safety of cheese. From a public health viewpoint, cheese is a very safe product and the consumer can be confident that a cheese is safe. The composition of cheese is characteristic of the variety and may be indicated on the consumer pack.

Table 17.4. Methods for acceleration of cheese ripening.

Method	Advantages	Disadvantages
Elevated temperature	No legal constraints; technically simple, no cost (perhaps saving)	Non-specific action, increased risk of spoilage
Indigenous enzymes	Low-cost, specific action, choice of flavour options	Difficult to ensure uniform incorporation into curd and to avoid regions of high or low activity; limited choice of useful enzymes, risk of continued enzymatic activity once desired effect is achieved, over-ripening; possible legal barriers
Modified starter cells	Easy to incorporate; natural enzyme balance retained	Technically complex, expensive
Genetically engineered starters	Easy to incorporate; choice of options	Possible legal barriers, may not be acceptable for different consumer markets
Cheese slurries and enzyme-modified cheese	Very rapid flavour development	High risk of microbial spoilage, final product has to be heat treated to inactive enzymes and microorganisms

The functionality of cheese includes such attributes as sliceability, adherence, meltability and stretchability. The first two of these are of concern mainly to cheese processors, while the last two relate mainly to the use of cheese as an ingredient.

The flavour of cheese is probably its most important attribute for most consumers but it is very much a matter of personal preferences between varieties and within a variety. The development of cheese flavour is discussed in section 17.6 and its assessment is discussed briefly in section 17.8.

Since the ripening of cheese, and hence its quality, is due to the activity of microorganisms and enzymes from four or five sources, it might reasonably be expected that it should be possible to produce premium quality cheese consistently by controlling these agents; however, in spite of considerable research and quality control efforts, it is not yet possible to do so with certainty. Inconsistency is due mainly to variations during manufacture, which it should be possible to avoid.

Many factors interact to affect the composition of cheese curd and hence the quality of the final cheese; an attempt to summarise these is shown in figure 2 of Fox and Cogan (2004). Some of these factors/agents can be manipulated easily and precisely while others are more difficult, or perhaps impossible, to control. The precise influence of many of the factors that affect cheese ripening and quality are not known and many of the factors are interactive.

The quality of cheese is influenced by its composition, especially moisture content, NaCl concentration (preferably expressed as % S/M), pH, moisture in non-fat substances (MNFS; essentially the ratio of protein to moisture) and % fat in dry matter (FDM). The effect of composition on the quality of Cheddar cheese has been the subject of a number of studies and the compositional parameters needed for optimal quality prescribed; for example, for premium-grade Cheddar, pH 4.95–5.10; S/M 4.0–6.02%; MNFS 52–56%; FDM 52–55%. High values for moisture and pH and a low salt content lead to flavour and textural defects, but within the prescribed zones, composition is not a good predictor of Cheddar cheese quality. Presumably, other factors, for example microflora, activity of indigenous milk enzymes, relatively small variations in cheese composition and probably other unknown factors, influence cheese quality but become dominant only under conditions where the principal determinants, moisture, salt and pH, are within appropriate limits. There appears to be no published work on the effect of composition on the quality of other cheese varieties, perhaps because the composition of the other principal varieties (Dutch, Swiss, Mozzarella) is less variable than that of Cheddar.

The concentration of calcium in cheese curd determines the cheese matrix and, together with pH, indicates whether a proper procedure was used to manufacture a specific cheese variety. As the pH decreases during cheese manufacture, CCP dissolves and is removed in the whey. The whey removed after cooking comprises 90–95% of the total whey lost during cheesemaking and contains, under normal conditions, about 85% of the calcium and about 90% of the phosphate lost from the cheese curd. Thus, the calcium content of cheese reflects the pH of the curd at whey drainage; there are strong correlations between the calcium content of cheese and the pH at 1 or 14 days and the amount of starter used (Lawrence *et al.*, 1984). Since the pH of cheese increases during ripening, the pH of

mature cheese may be a poor index of the pH of the young cheese and calcium concentration is probably a better record of the history of a cheese with respect to the rate of acidification than the final pH value. Reduction in CCP concentration by excessively rapid acid development also reduces the buffering capacity of cheese and hence the pH of the cheese will fall to a lower value for any particular level of acid development. The significance of calcium and phosphate to cheese quality has been reviewed by Lucey and Fox (1993).

The rate of ripening and cheese quality are strongly affected by temperature. Ripening at an elevated temperature is normally considered with the objective of accelerating ripening but it also affects cheese quality. Temperature has a strong influence, both desirable or undesirable, on the microflora and on the various enzymatic reactions. The rate of ripening can be delayed by reducing the temperature.

17.8 CHEESE FLAVOUR

Attempts to identify the compounds responsible for cheese flavour date from the early twentieth century. Initially, it was believed that flavour was due to a single or a few compounds, but is was soon realised that the flavour of cheese is due to the balance of several sapid and aromatic compounds – the component balance theory (Mulder, 1952; Kosikowski & Mocquot, 1958). Little progress on the identification of the key flavour compounds was possible until the development of gas chromatography (GC) in 1952. Several key compounds were identified in vacuum distillates of cheese and cheese headspace. The flavour profile has been extended gradually with improvements in GC, GC with sniffer ports and GC/MS (mass spectroscopy) (Le Quéré, 2004, 2011). To date, the electronic nose has not been very successful for the characterisation of cheese flavour, probably owing to the complexity of the flavour profile. Off-flavours, for example bitterness or rancidity, are more easily defined, because they are due to high levels of certain compounds, for example small hydrophobic peptides for bitterness or fatty acids for rancidity.

It is hoped that instrumental methods can be developed for the objective determination of cheese flavour but at present, this can best be done subjectively by a trained taste panel or consumer panel (Delahunty & Drake, 2004; Drake & Delahunty, 2011).

17.9 CHEESE TEXTURE

Cheese texture may be defined as a composite sensory attribute resulting from a combination of physical properties that are perceived by the senses of touch (tactile, including muscular and mouth-feel) and sight. Cheese texture is often considered to be synonymous with cheese rheology, which is not true. Rheology is the science of deformation or the relationship between stress and strain. The tactile properties of cheese depend on its rheological properties, which determine its behaviour to a stress or strain and is measured instrumentally (O'Callaghan & Guinee, 2004; Guinee, 2011a; Rohm & Jaros, 2011).

17.9.1 Measurement of cheese texture

For the consumer, texture characteristics based on tactility are perceived as the muscular response to the tension exerted on the cheese (e.g. during slicing, bending a slice, squeezing a piece between the fingers, biting, and chewing) or perceived visually. It can be measured directly using a trained sensory panel that may use different approaches, for example scoring the intensity of different tactile attributes (e.g. hardness, chewiness) or differentiating cheeses based on specific texture attributes (e.g. softness). However, owing to the difficulty and cost in assembling sensory panels, they are not used routinely to assess cheese texture. Instead, cheese texture is generally measured indirectly using instrumental rheological techniques, for example which measure the force required to compress, shear or penetrate a sample of cheese. Sample compression, which is used widely for hard and semi-hard cheeses, measures the force required to compress a sample of the cheese (cylinder or cube) to a predetermined degree (e.g. 75% of its original height) at a fixed rate (typically 20 mm/min) at a fixed temperature (typically 8°C). Analysis of the resultant force–displacement or stress–strain curves, often referred to as texture profile analysis (TPA), enables the determination of a number of rheological parameters, for example fracture stress, fracture strain, firmness and springiness, which are related to sensory textural characteristics, such as brittleness, sliceability, shredability, hardness and chewiness. Penetrometers and oscillation rheometers have been used to determine the viscosity of soft fresh cheeses and to demonstrate the influence of the various processing steps, for example heat treatment, homogenisation or cooling on texture properties.

17.9.2 Textural characteristics of different cheeses

The specific combination of properties that constitute the desired texture is variety dependent. For example, Feta cheese may be characterised as being visually white, slightly curdy/granular, moist and glossy, having few irregularly shaped holes, being short/crumbly (when squeezed or cut) and soft and delicate when eaten. Parmesan, on the other hand, is also crumbly, but has a dry and somewhat dull, light brownish, powdery and flaky appearance without openings, is hard to the touch, and has a mealy/grainy mouth-feel. In contrast to these cheeses, medium mature Gouda has a uniform, smooth waxy appearance, is firm and pliable, and has a slightly rubbery, chewy mouth-feel.

Texture is determined mainly by the interactive effects of composition and microstructure and macrostructure, where microstructure refers to the structural arrangement of compositional components (e.g. moisture, fat, protein, colloidal salts) in the curd, and macrostructure to the arrangement of curd particles or pieces/chips in the case of dry-salted cheeses such as Stilton, Cheddar or Cheshire.

In rennet curd cheeses, the microstructure is a matrix, consisting of a network of fused calcium phosphate *para*-casein micelles that occludes the fat, mainly in the form of coalesced globules or pools. Cross-linking of the caseins within the network is mediated by various interactions: calcium (attached to glutamate and aspartate), CCP (attached to serine phosphate groups), electrostatic and hydrophobic. The matrix may be considered somewhat analogous to a sponge, in which the *para*-casein network corresponds to the fabric and the fat globules to the air openings. The *para*-casein micelles in hard/semi-hard rennet curd cheeses are extensively dehydrated (~1.5 and 1.1 g/g *para*-casein in Cheddar and Parmesan, respectively) compared with the casein micelles in milk (~4 g/g casein).

17.9.3 Texture at the macrostructural level

For most cheese varieties, apart from Cottage cheese, which has discrete curd granules that are usually coated with a cream dressing, and Swiss-type cheeses, which have large gas holes (eyes), the macrostructure is essentially a continuum on a macro-scale, even though visible discontinuities may exist in the form of small holes, pockets, cracks, seams (curd granule junctions) or veins that are scattered throughout the continuum, which may be the face of a cut cheese loaf (block) or slice. Discontinuities can be observed visually in most cheeses at the end of manufacture, apart from cast cheeses (such as cast Feta), which are made by setting concentrated milk (liquid pre-cheese prepared by UF and very close textured hard cheeses such as Gouda and Swiss types. Discontinuities may occur as a result of factors that influence the contact area (surface) between curd particles, and the degree of fusion along the contact area when curd is moulded and pressed. The physical dimensions and geometry of the curd particles/pieces, and the packing arrangement (as influenced the curd size distribution and filling mechanism) determine the potential of the individual particles/pieces to fit together closely in a moulded cheese, while the microstructural and chemical characteristics, such as gross composition and pH, influence the viscoelasticity and tendency of individual particles to flow when pressed, and thereby the potential of particle surfaces to fuse and knit together, to create a homogeneous whole. Cheesemaking protocols for different varieties are designed to control the extent of discontinuities that occur between the curd particles/pieces that make up the final cheese. Some visible discontinuities are present in many cheeses at the end of manufacture, they may change in size or disappear during maturation, owing to biochemical changes that enhance the ability of the curd matrix to rearrange and flow, for example equilibration of salt and moisture; salt-induced hydration and solubilisation of the *para*-casein network at interfaces; proteolysis of the *para*-casein network; increase in pH (in some cheeses), which increases casein hydration.

Conversely, some cheeses may have a smooth closed texture at the end of manufacture and during the early stages of maturation but then develop eyes, irregularly shaped openings, cracks/slits/cavities on further ripening, generally as a result of the production and accumulation of gas at microstructural weak-spots in the curd. While eyes (e.g. in Emmental, Leerdammer, Gouda, Limburger, Havarti) and/or holes (e.g. in Tilsit, Limburger) of the desired size, distribution and surface sheen are an important quality attribute of some cheeses, slits are a quality defect (Martley & Crow, 1996; Daly *et al*., 2010).

17.10 PROCESSED CHEESE PRODUCTS

Heating cheese to a high temperature (80–100°C) under quiescent conditions, as in food preparation/service, generally results in some oiling-off and moisture exudation (especially acid curd cheeses). These effects, which occur to varying degrees depending on cheese type, reflect shrinkage and dehydration of the casein network and coalescence of fat. The possibility of increased cheese trade through the sale of heat-stable cheese products motivated the search to overcome the heat-induced destabilisation of cheese. In 1911, Swiss workers (Gerber and Stettler) produced a stable heat-treated Emmental cheese, known as Schachtelkäse, by the addition of a 'melting salt', sodium citrate, to the comminuted cheese before heating and shearing. Subsequently, it was found that other cheeses could be heat-treated successfully using citrates or phosphates, or blends of phosphates and citrates. The 'melting salts' are generally referred to as emulsifying salts (ES), which although they are not emulsifiers *per se*, they promote, with the aid of heat and shear, a series of physicochemical changes within the cheese blend which result in rehydration of the insoluble aggregated *para*-casein (matrix) and its conversion into an active emulsifying agent.

Processed cheese products (PCPs) are cheese-based products prepared by blending and melting/heating one or more natural cheeses, ESs, water and optional ingredients into a smooth homogeneous blend using heat and mechanical shear. The hot molten product is then moulded into a range of shapes and sizes suitable for retail (e.g. blocks, triangles, tubes, tubs) and food service (drums, slabs, sausages) applications. There are various types of PCPs, as

defined by national legislation, which differ with respect to types and levels of cheese and permitted (optional) ingredients and composition (Hickey, 2011). Similar to natural cheeses, different types of PCPs are available that vary in composition (e.g. ~40–70% moisture, ~10–20% protein, ~15–25% fat, 5.6–6.0 pH) and functional properties (colour, flavour, hardness, elasticity, shreddability, sliceability, meltability on heating). Among the advantages of PCPs over natural cheeses are storage stability and convenience; they represent 10–15% of total cheese consumption. Textbooks on PCPs include Meyer (1973), Berger *et al.* (1989), Zehren and Nusbaum (1992) and Tamime (2011).

The manufacture of PCPs involves the following steps:

- *Formulation*: selecting the different types and levels of ingredients required to give the desired product composition and characteristics.
- *Size reduction of the cheese*: by shredding, grating or mincing to maximise the surface area of the cheese and ensure uniform mixing with other ingredients.
- *Blending of ingredients*: to ensure homogeneity of all materials and uniform end-product quality.
- *Processing of the blend*: heating by direct or indirect steam injection typically to about 75–85°C for 1–5 min, usually in a batch cooker (kettle), while constantly agitating/shearing, in order to kill any potential pathogenic and spoilage microorganisms and to enhance the interaction between ingredients (e.g. ESs) and the physicochemical and microstructural changes required to transform the blend to a uniform molten mass.
- *Hot filling followed by controlled cooling and/or refrigeration*: to promote 'setting' of the hot molten blend to the desired consistency, via regulation of protein interactions and fat crystallisation.

17.10.1 Principles of manufacture

The protein in natural rennet curd cheeses occurs as calcium phosphate *para*-casein aggregates, formed by inter-protein interactions involving calcium (attached to glutamate and aspartate) and CCP (attached to serine phosphate groups), and hydrophobic interactions. The calcium content (mg/g *para*-casein) of rennet curd cheeses varies from about 15–18 mg/g casein in Blue-type to 35 mg/g in Emmental, owing to inter-variety differences in pH at rennet addition, scald temperature, pH at draining, pH and moisture content of the curd at moulding, and the degree of whey expressed from the moulded curd. Because of the relatively high level of calcium, the casein in natural rennet curd cheeses is insoluble. The protein in acid curd cheeses (e.g. Quark, Cottage cheese) occurs in the form insoluble casein/casein–whey protein aggregates, with hydrophobic, electrostatic and disulphide bonds (where the milk has been high heat treated) contributing to inter-protein association. The level of calcium in acid curd cheese is relatively low (e.g. ~10 mg/g casein) owing to the low pH of the gel at whey drainage (~4.7 compared with 5.8–6.4 for most rennet curd varieties). In contrast to rennet curd cheeses, the potential of protein in acid curd cheese to bind water is much greater than that of rennet curd cheese when the pH is readjusted to that of milk, i.e. about 6.7 (from 4.6), because of its full complement of caseins.

ESs affect the hydration of the calcium phosphate *para*-κ-casein, or casein and the hydrated protein emulsifies the fat released during the heating and shearing steps of the PCP manufacturing process. The principal ESs used in cheese processing are Na_2HPO_4 and trisodium citrate ($Na_3C_6H_5O_7$). ESs increases protein hydration via: (i) pH buffering which increases the pH from the typical value, about 5.2–5.6, of natural cheese to about 5.8–6.0 in the PCP blend, and stabilises it, and (ii) their calcium-sequestering ability, which removes of a large portion of the calcium and phosphate (~75%) attached to the *para*-casein or casein and replaces it by sodium. Decalcification of the calcium phosphate *para*-casein in rennet-curd cheeses at the elevated pH breaks intra- and inter-casein molecular cross-links and increases negative charge. Both these changes favour a more open reactive *para*-caseinate/caseinate conformation with superior water-binding capacity than natural cheese. The protein matrix of the natural cheese is transformed to a sodium caseinate or *para*-caseinate dispersion (sol) in acid- and rennet-curd cheeses, respectively.

17.10.2 Uses and characteristics of PCPs

PCPs are used both as retail products (blocks, slices, spreads and dips) and as cheese-based ingredients for food services (e.g. in sauces, quiche, sandwiches) and industrially prepared foods (ready-made meals, soups, sauces). In these applications, PCPs compete with other cheese products, including natural varieties, analogue/imitation cheese products, and blends of the latter. Hence, apart from being competitively priced, it is essential that PCPs have the desired functional attributes (e.g. texture, degree of melt on heating, stringiness, taste).

Being formulated products, PCPs can be easily customised to specific functionality and composition. Apart from gross composition, which can be manipulated easily, key factors that influence their functionality (Guinee *et al.*, 2004; Guinee, 2009; Tamime, 2011) include:

- natural cheese characteristics, for example pH, calcium-to-casein ratio, level of proteolysis, type and level of flavour compounds;
- the characteristics of optional ingredients used, for example protein composition, pH, mineral composition,

level of lactose and hydration properties of dairy protein products;
- the type and level of ESs, for example pH buffering and calcium-chelating effects;
- the manufacturing process, for example sequence of ingredient addition, processing conditions (time, temperature and shear) and cooling rate.

17.10.3 Cheese analogues

Cheese analogues (CAs) were developed in the 1950s as cheaper alternatives to natural cheese and PCPs. CAs may be classified as cheese substitutes or imitations; a cheese substitute is nutritionally equivalent to the PCP it simulates, while an imitation cheese is nutritionally inferior (Guinee, 2011b).

CAs are prepared by blending various vegetable oils/fats (e.g. hydrogenated palm or soya oils) and proteins (e.g. casein powders), water, ESs and other ingredients (e.g. cheese flavours, starches, hydrocolloids) into a smooth homogeneous blend with the aid of heat and mechanical shear. Protein, generally casein and/or *para*-casein, is the primary stabilising agent in CAs; the ES converts the protein to a functional form (e.g. sodium caseinate, sodium *para*-caseinate) that binds water, emulsifies oil during processing and forms a stable product.

While CAs differ from PCPs in that they do not contain cheese, both products share some similarities, such as inclusion of some of the same types of ingredients (e.g. ESs, flavours, milk proteins) in their formulations, similar manufacturing technology (heating and shearing in cookers), similar microstructures (concentrated oil-in-water emulsions stabilised by protein), the absence of a ripening period, the diverse range of textures, flavours, cooking properties and packaging formats, and their use as alternatives for natural cheese in a wide range of applications. The functional properties (e.g. sliceability, shreddability, cooking characteristics) of CAs are affected by many factors, including composition of the blend, characteristics of ingredients such as milk protein (initial solubility, pH, mineral composition, casein–whey protein ratio), fat/oils, starches (amylose-to-amylopectin ratio, type and level of modification), types and level of ESs (degree of polymerisation, citrates or phosphates) and processing conditions.

Compared to PCPs, CAs may be preferred as ingredients in formulated or assembled food products (e.g. cheese flavourings, cheese sauce toppings, frozen pizza, prepared meals) primarily owing to the lower cost. The main factors contributing to the relatively low cost of CAs are the inclusion of vegetable oils rather than milk fat, and the partial replacement of protein (mainly casein) by starches which may be included at a level up to 5% (w/w) or higher.

17.11 CHEESE AS A FOOD INGREDIENT

Cheese has long been used in the home and catering establishments as an ingredient, along with other foods and condiments, in the preparation of quiche, lasagne, cheesecake, toasted sandwiches, omelettes, sauces or gratins. It is used as an ingredient in both the food service (catering) and industrial cheese sectors (Guinee & Kilcawley, 2004; Guinee, 2011c). In the former, cheese is used extensively in the preparation of ready-to-eat meals or snacks (e.g. pizza, cheeseburgers, tortillas, macaroni and cheese, tacos with cheese, quiche, lasagne, toasted sandwiches, sauces, salads, desserts) sold mainly through restaurants, fast-food outlets and delicatessen counters. Industrial cheese refers to that which is used by the industrial sector for the commercial manufacture of assembled foods (e.g. frozen and chilled pizza, prepared sandwiches, cheese salads), formulated foods (e.g. gratins, prepared meals, PCPs, co-extruded products, cheese cake, dairy desserts) and food ingredients (e.g. cheese sauces, cheese powders, enzyme modified cheeses, ready-to-use grated/shredded cheeses, dried grated cheeses, freeze-dried cheese pieces). While formulated and assembled cheese-containing foods are sold principally through retail and food service outlets, cheese as a food ingredient is used mainly by the manufacturers of formulated foods (e.g. soups, dried cheese sauces, dehydrated potato mixes, infant meals, pasta dishes, snack coatings, bakery products) and to a lesser extent by the catering or food service sector in the preparation of culinary dishes.

On the US and European cheese markets, which together account for about 75% of total cheese (~13 Mt), it is estimated that cheese used by the ingredient and food service sectors accounts for at least about 30% of all cheese consumed, but varies from about 60% in the USA to 45–50% in UK and Germany, to close to zero in some East European countries (Voorbergen, 2011). It represents the fastest growing sector of cheese consumption, but the manufacture of ingredient cheese requires a relatively high research and development input to develop customer-specific solutions or to create novel attributes (e.g. defined size reduction characteristics, heat stability, flow resistance, heat-induced fluidity, heat-induced congealing) that differentiate products and attract added value.

Cheese products used as food ingredients are supplied in different formats: natural named-variety cheeses, customised generic cheeses, PCPs (e.g. slices, sauces, dips) and analogue or imitation cheese products (CAs), and blends of the latter. Natural named-variety cheeses must comply with specific national or international standards (e.g. Codex Alimentarius, Code of Federal Regulations) for manufacturing protocols. In contrast, natural generic cheeses are

not limited by these standards and are generally made to customer specifications regarding composition and functionality. PCPs and CAs can be easily customised to specific functionality and composition (see section 17.10). However, both of these products differ structurally from natural cheeses and can lack some of the defining characteristics of natural hard, semi-hard cheeses (e.g. chewiness, longness and stringiness available from cheese such as Mozzarella, Cheddar, Emmental).

As an ingredient, cheese is generally required to provide different flavour, texture and cooking characteristics. The flavour of cheese is a key quality attribute in most applications in which cheese is used as an ingredient,. The vast array of natural cheese varieties provides a diversity of flavours (e.g. savoury, piccante, tangy, nutty, sweetish, salty) that contribute to the foods in which they are used.

PCPs, which are used extensively as ingredients in foods (especially sauces, burgers, sandwiches), are also capable of imparting a range of flavours owing to the inclusion in the formulation of different types and quantities of natural cheeses, enzyme-modified cheeses (EMCs), hydrolysed butter oil, and flavour condiments. EMCs are essentially pasteurised cheese pastes with intense flavours that simulate those of specific natural cheeses (Kilcawley *et al*., 1998). Key steps in their manufacture include the formation of a paste by blending fresh cheese curd, water and ESs and heat-treating to inactivate microorganisms and enzymes in the cheese; adding enzymes and starter cultures (optional) to the cooled paste, incubating the blend, typically at 25–35°C, usually for a few days, until the correct flavour profile and intensity has developed; re-pasteurising the blend to inactivate the added enzymes and/or cultures, and, thereby, stabilise the flavour. The use of EMCs in CAs contributes flavour when used as an ingredient; however, CAs are generally used more for their physicochemical properties on cooking than for their flavour.

In ingredient applications, cheese products are used mainly in size-reduced formats (e.g. shredded, diced or grated cheese, pieces, slices) that are convenient to use. During size reduction operations, cheeses are subject to a combination of shear and compressive stresses that result in fracture. The behaviour of cheese when subjected to different size reduction methods constitutes a group of important functional properties which are related mainly to rheological characteristics. For example, 'long' cheeses that are moderately firm and have a high fracture strain (e.g. low-moisture Mozzarella, Emmental, Gouda) are generally better suited to applications requiring cheese slices, cubes or shreds than crumbly cheeses with a low fracture strain (e.g. Parmesan, Cheshire, Feta) that give jagged fracture surfaces.

In most applications as an ingredient, cheese is heated, and consequently, the characteristics of the cooked cheese (e.g. meltability, flow, oiling-of, succulence, viscosity/fluidity, chewiness) are important. Depending on the application, one or more of these characteristics are required. For example, in pizza, the stretchability, stringiness, chewiness, moderate flow and slight oiling-off of *pasta filata* varieties (e.g. Mozzarella, Provolone, Kachkaval and string cheese) are key quality attributes. Conversely, the softness (non-chewy), high flowability and more extensive oiling-off of mature Cheddar and Raclette cheeses are better suited to toasted sandwiches.

17.12 CHEESE PRODUCTION AND CONSUMPTION

World production of cheese is about 17 Mt per annum (~35% of total milk production) and is increasing at a rate of 2–3% per annum. Europe, with an annual production of about 8 Mt, is the principal producing region, followed by North America (Table 17.5). Cheese consumption, which varies widely between countries (Table 17.6), has increased consistently in most countries for which data are available; along with fermented milks, cheese is the principal growth product within the dairy sector. There are many reasons for the increased consumption of cheese, including a positive dietary image, convenience and flexibility in use and a great diversity of flavours and textures. Cheese can be regarded as the quintessential convenience food: it can be used as a major component of a meal, as a dessert, as a component of other foods or as a food ingredient; it can be consumed without preparation or subjected to various cooking processes. The most rapid growth in cheese consumption in recent years has been as a food ingredient.

17.13 CLASSIFICATION OF CHEESE

There are at least 1000 varieties of cheese, about 500 of which are recognised by the International Dairy Federation. For various reasons, a number of attempts have been made to classify cheeses into meaningful groups. Traditional classification schemes have been based principally on moisture content, i.e. extra-hard, hard, semi-hard/semi-soft or soft. Although used widely, this scheme suffers from serious limitations since it groups cheeses with widely different characteristics, for example Cheddar and Emmental are classified as hard cheeses although they have quite different textures and flavours, are manufactured by very different technologies and the microbiology and biochemistry of their ripening are very different. In addition, cheeses traditionally developed a rind through which moisture evaporated. Hence, the composition of cheese changes as it ages and there is a moisture gradient from the surface to the centre; the moisture content of long-ripened rinded

Table 17.5. Production of cheese (1000t), 2008.

Europe	
Austria	137
Belgium	67
Bulgaria	63
Czech Republic	109
Denmark	324
Estonia	36
Finland	107
France	1765
Germany	2032
Greece†	230
Hungary	73
Ireland	140
Italy	1064
Latvia	40
Lithuania	107
Netherlands	724
Norway	84
Poland	637
Portugal	57
Romania	65
Russia	425
Slovakia	34
Slovenia	20
Spain	119
Sweden	114
Switzerland	179
UK	339
Ukraine*	346
North, Central and South America	
Argentina	491
Brazil*	580
Canada	402
Chile	65
Mexico*	154
Uruguay*	48
USA	4463
Africa, Asia and Oceania	
Australia	343
Iran*	230
Israel*	112
Japan*	125
New Zealand	355
South Africa*	42

*Based on data from International Dairy Federation (2008), Bulletin 432.

†Based on data from Barry Wilson's Dairy Industry Newsletter (2009), **21** (13).

Source: based on data from the Dutch Dairy Board.

Table 17.6. Consumption (kg per head per annum) of cheese, 2007.

Greece*	30.0
France	24.3
Iceland	23.5
Norway	23.5
Germany	22.2
Switzerland	22.2
Italy	20.5
Finland	19.1
Austria	18.8
Sweden	18.4
Netherlands	17.3
Czech Republic	17.0
Denmark†	16.5
Israel	16.5
USA	16.0
Belgium†	14.6
Canada	12.6
Luxembourg†	12.6
UK	12.2
Australia	11.9
Argentina	11.2
Poland	10.7
Portugal*	10.5
Hungary*	10.4
Slovakia†	10.2
Estonia	10.0
Slovenia†	9.4
Croatia	8.2
Spain	7.2
Russia*	6.2
Ireland†	6.1
New Zealand	6.1
Bulgaria†	5.6
Lithuania†	4.9
Chile	4.0
Latvia	3.7
Mexico†	2.2
Japan*	2.0
South Africa	1.6
Ukraine	1.1

*2006.

†Based on data from the Dutch Dairy Federation.

Source: based on data from International Dairy Federation (2008), Bulletin 432, unless otherwise indicated.

cheese may decrease by 5–10% during ripening. The composition-based scheme is made more discriminating by including information on the source of the milk, the coagulant, principal ripening microorganisms and the cook temperature. Based on the method of milk coagulation, cheeses may be divided into four superfamilies:

1. rennet-coagulated cheeses (most major cheese varieties);
2. acid-coagulated cheeses (e.g. Cottage, Quark, Cream);
3. heat/acid coagulated (e.g. Ricotta);
4. concentration/crystallisation (e.g. Mysost).

Owing to the great diversity of rennet-coagulated cheeses, these can be classified further based on the characteristic ripening agent(s), for example internal bacteria, internal mould, surface mould or surface smear (bacteria), or manufacturing technology; such a scheme is shown in Fig. 17.7.

17.14 CHEESE AS A SOURCE OF NUTRIENTS

Cheese is an important source of general nutrients and, in addition, has some specific beneficial effects. Although the composition of cheese (Table 17.7), and hence its nutritional value, varies considerably, cheese generally is a rich source of fat, protein, fat-soluble vitamins, calcium and phosphate. Some varieties of cheese contain a high level of fat but most varieties have a higher protein/fat ratio than other major foods. Cheese is especially significant in the human diet in Europe, North America, Australia and New Zealand. In France, with an annual consumption of 24.3 kg cheese per caput (Table 17.6), cheese contributes about 13% of the recommended energy (2000 kcal/day), about 30% of the daily protein requirement for men (~56 g/day) and 50% of the recommended calcium intake (1000 mg/day) in the human diet.

Since the principal cheese-producing countries are relatively affluent with a varied and nutrient-rich diet, cheese is not essential and can be readily replaced by fresh or fermented milk, meat or fish. However, inclusion of cheese in the diet increases diversity and variety. It is probably true that most people consume cheese because of its organoleptic and functional attributes or its convenience and flexibility of use. The nutritional aspects of cheese have been reviewed by Renner (1993) and O'Brien and O'Connor (2004).

17.14.1 Fat in cheese

Cheese is frequently regarded as a high-fat food but its fat content varies from about zero (Cottage) to about 38% w/w (Danish Blue) on a wet-weight basis, and hence choices are available. A 50-g serving of Cheddar cheese contains about 17 g of fat, which is a substantial proportion of the fat intake in affluent Western societies; a typical Western diet provides about 2000 kcal per day, about 40% of which is derived from about 88 g of fat. Cheese fat generally contains approximately 65% saturated, 30% monosaturated and 5% polyunsaturated fatty acids. Attempts to reduce the fat content and proportion of saturated fatty acids in cheese have had only limited success because of reduced organoleptic and functional properties.

The cholesterol content of cheese is a function of its fat content and ranges from about 10 to 100 mg per 100 g, which is low compared with many other animal-derived foods. From a nutritional viewpoint, the cholesterol in cheese is less important than the level of saturated fatty acids.

A positive characteristic of ruminant lipids (milk and adipose tissue) is a relatively high level of conjugated linoleic acid (CLA), which is considered to have antioxidative and anticarcinogenic properties. Milk and cheese are important sources of CLA, which can be increased by increasing the level of linoleic acid in the animal's diet.

17.14.2 Protein in cheese

Cheese contains 4–40% high-quality protein (Table 17.7). For most cheese varieties (rennet- or acid-coagulated), the vast majority of the protein is casein, which is deficient in sulphur-containing amino acids, resulting in a nutritional value of 91–97 compared to 100 for total milk protein (Renner, 1993). The incorporation of whey proteins in cheese, as in heat acid-coagulated varieties and some cheeses made from UF retentate, increases the nutritional value of its proteins. The proteins in cheese are very digestible, being more digestible than milk protein owing to proteolysis during ripening.

There is very good evidence that cheese has good cariostatic effects, mainly by hardening tooth enamel damaged by acids in some foods or produced in plaque. Thus, cheese is important for teeth development, both by providing calcium and phospahte for initial development and by reducing cariogenesis.

The caseins contain several biologically active peptides encrypted within the sequences of the caseins, some of which are released during ripening, especially angiotensin-converting enzyme inhibitor, casomorphins and phosphopeptides (FitzGerald & Meisel, 2003). It has been proposed that phosphopeptides can serve as good carriers of nutritionally important metals for food supplementation, but further work is required to confirm this.

In some varieties, especially surface smear-ripened varieties, extensive decarboxylation occurs, and some of the resulting amines, especially histamine and tyramine, are biologically active but cause problems only in patients using monoamine oxidase inhibitors.

17.14.3 Lactose

Fresh rennet curd cheese contains about 1% lactose, which is catabolised during ripening. Washed acid curd cheeses, for example Cottage, contain no lactose. Thus,

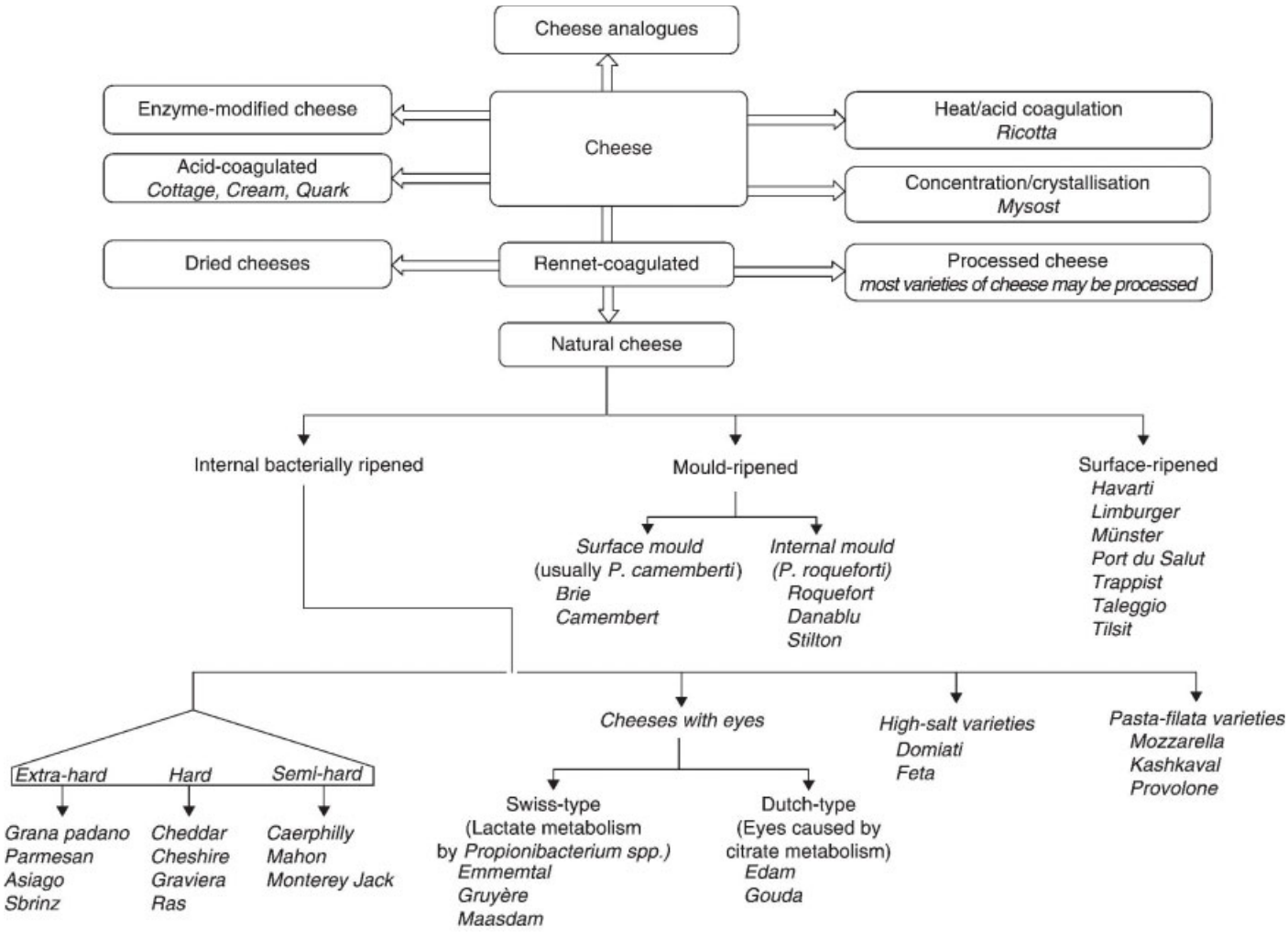


Figure 17.7. A scheme for the classification of cheese. Reproduced from Fox *et al.* (2004), with permission of Elsevier.

Table 17.7. Approximate composition of selected cheese varieties.

Variety	Moisture (%, w/w)	Protein (%, w/w)	Fat (%, w/w)	Sodium (mg/100 g)	Calcium (mg/100 g)
Brie	48.6	19.2	26.9	703	184
Camembert	50.7	20.9	23.7	842	388
Cheddar	37.2	25.4	33.1	710	730
Reduced-fat Cheddar	43.0	33.4	17.0	745	934
Cheshire	38.5	24.2	31.9	710	473
Cottage cheese (creamed)	79.1	13.8	3.9	393	94
Danish Blue	43.0	18.4	37.3	1297	465
Edam	43.8	26.0	25.4	785	731
Emmental	35.7	28.7	29.7	256	936
Feta	56.5	15.6	20.2	1116	492
Fromage frais	77.9	6.8	7.1	?	?
Gouda	40.1	24.0	31.0	819	721
Gruyère	33.6	27.3	34.5	467	666
Mozzarella	49.8	25.1	21.0	589	710
Parmesan	30.6	34.9	26.0	1108	1097
(Block) Processed cheese	49.1	18.3	23.3	1351	531
Ricotta	73.2	11.3	10.3	141	272
Roquefort	41.3	19.7	32.9	1500	560
Stilton	40.5	21.6	33.7	1360	420

Sources: based on data from Posati & Orr (1976), Kosikowski & Mistry (1997), Fenelon & Guinee (1999), Guinee *et al*. (2000) and unpublished data.

all types of cheese can be consumed with impunity by lactose-intolerant people.

17.14.4 Inorganic elements

Rennet-coagulated cheeses are rich sources of calcium and phosphate but acid-coagulated varieties contain much lower levels. Cheese makes a significant contribution (up to 50%) to the dietary intake of calcium in Western societies. Calcium is important for the development and maintenance of bone and teeth and a high intake in childhood and early adulthood reduces the risk of osteoporosis in later life.

Milk and cheese are poor sources of iron, which is a potent pro-oxidant, and fortification of milk with iron leads to the development of lipid oxidation (oxidative rancidity). However, it has been reported (Zhang & Mahoney, 1991) that Cheddar and processed cheese can be fortified with iron without ill effects, possibly because cheese has a low redox potential (–200 mV).

As discussed in section 17.4.8, most cheeses are salted, which has important effects on cheese ripening and quality. The typical sodium content in selected cheeses is shown in Table 17.7. Processed foods are major contributors of dietary sodium but substantial quantities may be added during cooking and by individual consumers at the table; sodium strongly affects hypertension. At the average level of European consumption (~15 kg per annum) cheese is not a major contributor to dietary sodium (~12% of the RDA) but efforts are being made to reduce the level of NaCl in cheese (see section 17.4.8).

17.14.5 Vitamins

The fat-soluble vitamins in milk are transferred completely to cheese, which can be a good vehicle for supplementation. However, most of the water-soluble vitamins in milk partition into the whey, and hence all cheeses are poor sources of these vitamins, although some biosynthesis of water-soluble vitamins by bacteria occurs during ripening.

17.15 CONCLUSIONS

Through increased knowledge of the chemistry, biochemistry and microbiology of cheese, it is now possible to consistently produce high-quality cheese, although this is not always achieved owing to failure to control one or more of the key parameters that affect cheese composition and ripening. Milk is a variable raw material and although it is possible to eliminate major variations in the principal milk constituents some variations persist. Variability in milk composition can also be compensated by manipulating process parameters in the cheesemaking process. Most large factories operate on a strict time schedule and hence subtle process manipulation on an individual vat basis

may not be possible. Therefore, strict control of milk composition and starter activity are critical.

From a microbiological viewpoint, the milk supply to modern cheese factories is of very high quality and after pasteurisation is essentially free of bacteria. In modern factories where enclosed vats and other equipment are used, the level of contamination from the environment is very low; cheese curd containing less than 10^3 NSLAB/g at 1 day is normal. However, these adventitious NSLAB grow to about 10^7 cfu/g and dominate the microflora of long-ripened cheese. Although the significance of the adventitious NSLAB in long-ripened cheese is unclear, it appears desirable to control them, either by eliminating or standardising them. It is not possible to eliminate NSLAB, even in cheese made on a pilot scale under aseptic conditions. Their growth can be prevented by ripening at about 1°C but the overall ripening process is reduced to an unacceptable rate. Outcompeting endogenous NSLAB by an adjunct *Lactobacillus* culture is possible and is being used commercially.

Although it is now possible to avoid major defects in cheese produced using modern technology, further research on the biochemistry of cheese ripening is required to enable the process of cheese manufacture and ripening to be refined to an extent that will allow the consistent production of premium quality cheese.

A key to successful cheesemaking is a good reliable starter, both from the viewpoint of reproducible acid production and subsequent ripening. If properly managed, modern starters are generally satisfactory and their performance is being improved progressively.

The use of starter adjuncts, usually mesophilic lactobacilli, for some varieties, especially Cheddar, is increasing, with the objective of intensifying and modifying cheese flavour, accelerating ripening and perhaps controlling adventitious NSLAB and thus standardising quality.

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18
Butter, Ghee, and Cream Products

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18.1 INTRODUCTION

Butter, ghee, and cream are complex biological dairy products composed of mainly milk fat and other minor components, such as water, minerals, vitamins, and enzymes. The milk fat components are highly concentrated in ghee followed by butter and cream, and much of the protein fractions are lost in the preparation of ghee, and few beneficial components are generated during the fermentation of cream, butter, and ghee. These dairy products are highly nutritive and are rich in components that are beneficial for health, such as milk fat globule membrane (MFGM), conjugated linoleic acid (CLA), and fatty acids. In addition to its nutrient value when consumed in the diet, MFGM also contains many bioactive components that are beneficial for health (Mather, 2000). Phospholipid compounds in MFGM play various roles, including cell proliferation, apoptosis, signal transduction, blood coagulation, and neuronal signaling in the human body (Pettus *et al*., 2004). In addition, MFGM sphingolipids also show antibacterial and cholesterol-lowering activities in the human body (Rombaut & Dewettinck, 2006). Another health beneficial component is CLA, which enhances the quality of butter during storage (Baer *et al*., 2001). In addition, some dairy starter bacteria like *Propionibacterium* can produce CLA by converting linoleic acid *in vitr*o (Jiang *et al*., 1998). However, such conversion in fermented cream and butter is yet to be studied.

The health benefits of cream and butter are well known in a world where Western and European food types are increasingly consumed; however, health benefits of ghee are still being discovered. Ghee is well known on the Indian subcontinent and it was produced in ancient India as far back as 1500 BC (Achaya, 1997). The major use of ghee is in frying and dressing foods, and it is considered a sacred article in some religious rites (Rajorhia, 1993). Ghee in pure form is used to feed children because of its therapeutic value, and is mixed with honey and used as an aphrodisiac. Ghee is considered to be fairly stable due to the low water content and high antioxidative properties. Ghee is also rich in CLA, which shows anticarcinogenic effects (Sserunjogi *et al*., 1998).

Even though cream, butter, and ghee are rich in milk fat components beneficial for health, they are also rich in saturated and unsaturated fatty acids, triacylglycerol, and cholesterol (Jensen, 2002). Since some of the saturated fats are considered to contribute to disease such as atherosclerosis, their role in human health is quite controversial (Berner, 1993). Some fatty acids add a certain specific flavor to butter, such as butyric acid, but they also have health benefits, such as anticancer properties. Some research has also been conducted to remove cholesterol and to improve the health benefits of cream, butter, and ghee. Recent research in dairy products has shown that milk fat components possess some functions beneficial for health in addition to the basic nutritive value, and various health beneficial biological compounds have been detected in milk, but less is known about milk products, such as butter, ghee, and cream. The

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purpose of this chapter is to focus on the manufacture and nutrition of butter, ghee, and cream with major emphasis on their health beneficial compounds.

18.2 MANUFACTURE OF BUTTER, GHEE, AND CREAM PRODUCTS

18.2.1 Butter

Butter is a water-in-oil emulsion with a minimum fat content of 80%, in which water content should not exceed 16% and non-fat milk solids generally constitute 2%. There is a substantial annual consumption of butter worldwide and world production of butter is as high as 4.1 million tons per annum (Mortensen, 2011). Butter products are broadly classified as sweet cream unsalted, sweet cream salted, cultured unsalted, cultured salted butter, or traditional sour cream butter. For manufacturing commercial butter "continuous buttermaking" is typically used but the traditional small-scale on-the-farm method is the batch procedure, using rotational or upside-down churning at the right temperature. The continuous procedure is called the Fritz method after its inventor. Modern continuous butter machines are capable of producing 500–15000 kg of butter per hour (Renner, 1983; Codex Alimentarius, 2003; Mortensen, 2011).

As shown in Fig. 18.1, pasteurized cream is carried over to the churning section where phase inversion to the water-in-oil form takes place immediately. Rotation of the beater while churning is maintained at an optimum speed and continues until the diameter of the butter grains reaches 2–4 mm. The churning temperature is generally controlled at around 12°C to ensure that as much fat as possible in the cream is converted to butter. Churning is regarded as optimal when buttermilk shows a milk fat content of not higher than 0.5%. Buttermilk is drained away from the butter grains by a horizontal and slow rotating sieve drum in the separation section. The optimum temperature of the butter should be maintained at 5–7°C, and this is done by recirculating chilled buttermilk (Walstra *et al.*, 1999; Mortensen, 2011).

As the butter grains accumulate, larger lumps form which are subsequently transported to the working section where butter is kneaded to expel more water and to achieve the desired shape and texture. In the final part of the first working section, the starter culture is mixed with lactic acid concentrate and salt suspension is added if the goal is to produce cultured salted butter (pH ~5.2). With a view to reduce air content and to give a finer texture, butter undergoes a vacuum treatment between the two working sections. The second working section operates at a higher speed for proper mixing of salt and starter culture. The working sections are kept cool (14–16°C) with circulating chilled water as this temperature determines both the size and the composition of the continuous fat phase. After passing through the working sections, butter is kept in the balance tank for at least one hour to cool down to refrigerated temperature before conveying to the packaging section. In the continuous buttermaking process, the balance tank serves as a buffer between churning and packaging (Munro, 1986; Mortensen, 2011).

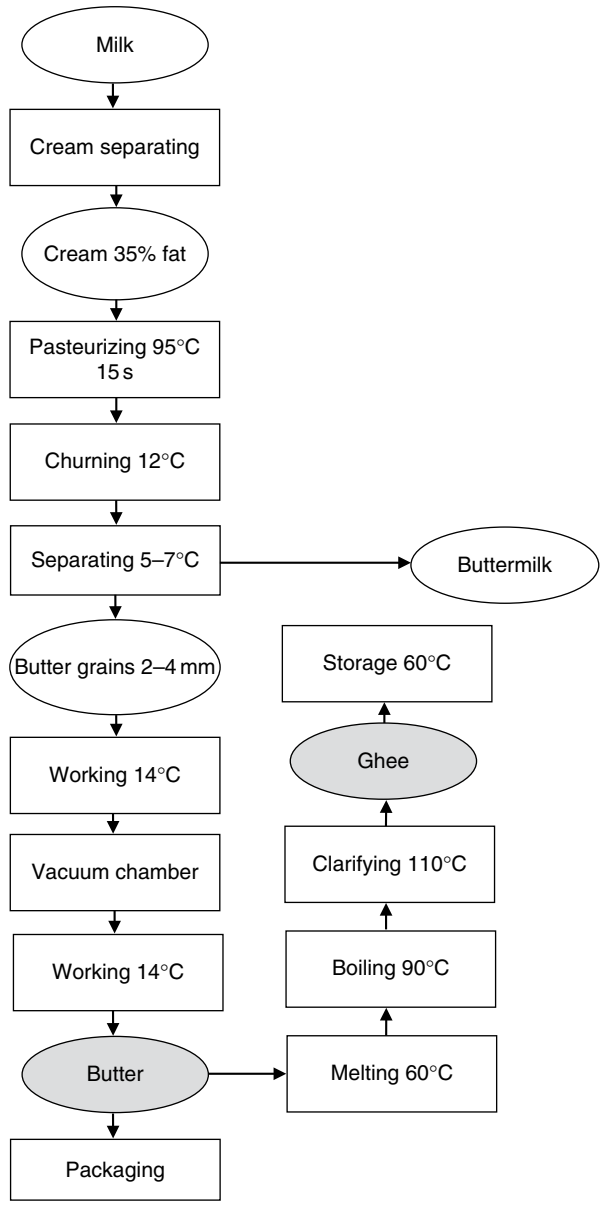


Figure 18.1. Flow diagram for manufacturing butter and ghee.

18.2.2 Ghee

Ghee is regarded as the Indian version of clarified butterfat, mostly produced from cow milk, buffalo milk, or mixed milk (Rajorhia, 1993; Sserunjogi *et al*., 1998). Its origin dates back to prehistoric Indian civilization as far as 1500 BC. In some Middle Eastern countries similar kinds of products are usually made from goat, sheep, or camel milk and are commonly known as *maslee* or *samn*. In Iran, ghee is called *rogham* (Urbach & Gordon, 1994). Ghee is specified as containing a minimum 99.6% milk fat and 0.4% free fatty acid with no more than 0.1% moisture (Codex Alimentarius, 2006). In India, the annual production of ghee has surpassed 800 000 tons, most of which is utilized for culinary purposes.

Ghee manufacture in India is largely based on the indigenous milk butter method, but the creamery butter method is now the most efficient procedure, and the majority of dairies today use this method. This process starts with melting the butter at 60°C, which is eventually delivered to the steam pressure boiler. As shown in Fig. 18.1, the temperature in the boiler is raised to 90°C and kept constant as long as moisture is being released. The scum floating on top is removed regularly using a perforated ladle. When the moisture has been driven off, the temperature must be increased gradually. The end point is indicated when fine air bubbles appear on the surface and curd particles start to turn brown. Typical ghee aroma is produced at this point. Afterwards ghee is channeled into a storage tank through a clarifier and is cooled to 60°C (van den Berg, 1988; Abhichandani *et al*., 1995; Sserunjogi *et al*., 1998). To protect against tampering and allow convenient transportation, ghee is typically packed in tin cans with capacities ranging from 20 g to 15 kg. In some cases, polymer-coated cellophane, nylon-6, polyester, food grade PVC, or different types of laminates are used for ghee packaging. The quality of ghee deteriorates if rancidity develops. It has been reported that at ambient temperatures ghee can be stored for 6–8 months; however, a longer shelf-life up to 2 years has also been observed (van den Berg, 1988; Rajorhia, 1993; Sserunjogi *et al*., 1998). For maintaining proper quality during storage it has been suggested that ghee should be stored at below 20°C.

18.2.3 Cream

Cream is an emulsion of fat globules in milk serum. It is separated from whole milk based on the difference in the specific gravity of the fat globules and aqueous phase. Depending upon fat content, there are a variety of creams found around the world, for example coffee cream (10–15% fat), half cream (10–12% fat), cultured cream (10–40% fat), single cream (20% fat), whipping cream (35% fat), double cream (45% fat), and clotted cream (55–60% fat). Among these products, coffee cream (10–15% fat), cultured cream (10–40% fat), and whipping cream (35% fat) are the most prominent. Despite having a common manufacturing procedure up to cream separation from whole milk, each type goes through a specific pathway in order to be turned into the final products (Renner, 1983; Hoffmann, 2011).

Fat globules usually float in milk due to their lower density (~0.9 g/mL) than milk serum (~1.0 g/mL). When whole milk is transferred to the cream separator, the built-in centrifuging system accelerates the rate of separation. Centrifugal force causes the denser aqueous phase to move outward at a higher velocity and is transferred to the skim milk outlet while the fat globules gather around the axis and are channeled out to the cream outlet. Optimum temperature in the separator is maintained between 50 and 60°C so as to resist protein denaturation, maintain the viscosity level, and protect the fat globule membrane, which ensure the maximum degree of separation. Generally, 35–45% fat in cream and 0.05% fat in skim milk are obtained from the separation. As shown in Fig. 18.2, when the cream is separated, the fat is standardized by addition of skim milk or cream to achieve the characteristic fat content of the specific type of cream (Hoffmann, 2011).

18.2.3.1 Coffee cream

Coffee cream is one of the most widely consumed cream products; it is mainly used as a whitening agent in coffee making, and in addition it gives a pleasant texture and flavor to the coffee. The fat content in coffee cream is the lowest among the cream products, containing 10–15% fat. This product is manufactured for a long storage period, usually applying flow sterilization in an ultra-high temperature (UHT) plant (Towler *et al*., 2003).

After separating from whole milk, the standardized cream is transferred to high-temperature short-time pasteurization (90°C for 15 s) before cooling to 6°C. Cream is then stabilized with sodium phosphate or sodium citrate suspensions. Fat globules with a diameter of 0.2–2.0 μm after homogenization indicates long storage, higher coffee stability, and excellent whitening effect. To ensure this quality, single- or double-stage upstream homogenization is carried out before flow sterilization at the UHT plant (130°C), followed by double-stage downstream homogenization with a total pressure of 20 MPa. After cooling to 25°C, the cream is filled aseptically into glass bottles or paper cartons with an aluminum foil wrapping (Hoffmann, 2011). In the USA there is a popular coffee cream called "half and half" (10% fat) packaged in small plastic cartons designed to serve one cup of coffee that does not require refrigeration as it has been pasteurized and refrigerated or sterilized using the UHT method.

18.2.3.2 Cultured cream

Cultured creams, also commonly known as sour creams, have a number of applications in the food industry as

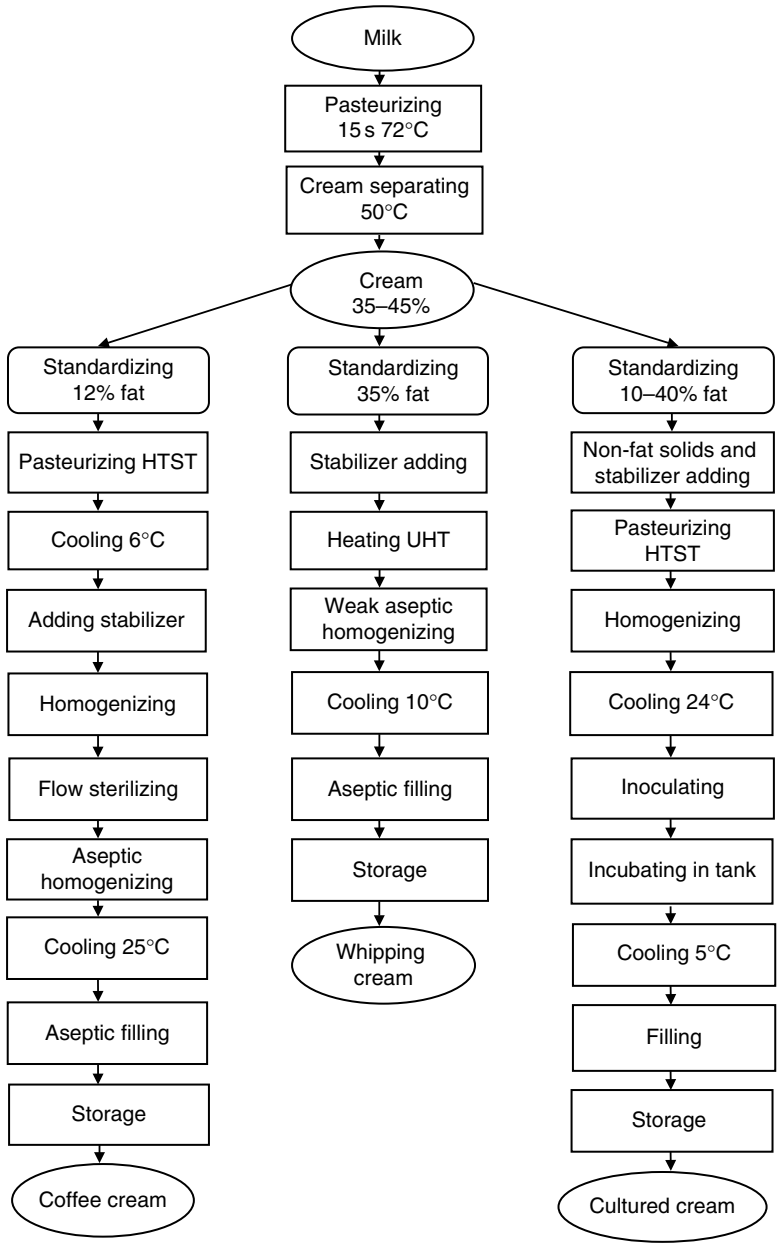


Figure 18.2. Flow diagram for manufacturing of coffee, whipping, and cultured creams.

condiments. They are used as dressings in salad, in cake mix, biscuits, doughnuts, and cookies, and are popular as desserts with toppings of fruit and sugar; they are also used for making sour cream butter. Cultured creams are manufactured with a fat content ranging from 10 to 40%. To achieve the desired fat content, cream is standardized. Addition of some non-fat dry matter and stabilizers improves the texture and prevents syneresis. As illustrated in Fig. 18.2, standardized cream is heat treated at 90–95°C for 15 s and homogenized for better textural properties. Souring characteristics of cultured creams are attributed to inoculation with lactic acid bacteria. Incubation is carried out in a large tank when the optimum condition for incubation is essentially maintained at 20–24°C for 14–24 hours.

When the pH is nearly 4.5, rapid cooling to 5°C stops microbial growth. A cultured cream is considered good quality when it is viscous, creamy, and has a good texture (Towler *et al*., 2003; Hoffmann, 2011).

18.2.3.3 Whipping cream

Whipping cream is categorized as having 30–40% milk fat with a prolonged shelf-life. The major applications of whipping cream include topping for coffee, ice cream, pie, cakes, pastries, puddings, and other desserts. For manufacturing whipping cream, heat treatment varies widely but, in general, a temperature of 80°C or higher is used, while in some cases a temperature even higher than 135°C is applied. During homogenizing, a low pressure (1.0–3.0 MPa) is recommended for preserving the whipping properties. Figure 18.2 indicates that as the cream undergoes heat treatment and homogenization, it needs to be cooled down rapidly, to as low as 4°C, and after cooling the temperature should not rise above 8°C. The chilled cream is channeled into a ripening tank through the bottom, where it is kept at 6°C for 24 hours. An agitator inside the ripening tank provides gentle mixing and helps crystallization before retail containers are filled (Hoffmann, 2011). During the whipping process, continuous and gentle agitation incorporates air into the cream. As the stirring proceeds, the air bubble becomes smaller and fat globules interact to form a stable network. There are several factors associated with stable whipping properties, including fat content, protein content, processing conditions, addition of stabilizer, and emulsifier (Bruhn & Bruhn, 1988). Whipping creams are generally supplied in small bottles, plastic cups, or large cans, and often in aerosol cans for convenience.

Butter, ghee, and cream are also made from other species, such as buffalo, goat, sheep, camel, yak, and reindeer, depending on the animal source in the various countries. Buffalo are abundant in India, Pakistan, Sri Lanka, and Bangladesh; goat and sheep in Mediterranean countries; camels in Arab countries, India, Pakistan and North Africa; reindeer in Eurasia; and yak in China, Mongolia, Nepal and India, where milk and dairy products are also produced from these animals (Park & Haenlein, 2006). However, the characteristic quality of the butter, ghee, and cream made from the different animal sources differs from the cow milk products and this is mainly due to the fat globule size in the milk and the position of fatty acids in the milk source. Butter made from sheep milk is less preferred due to its firmer structure, low iodine value, and its whiter color due to lower carotenoids in te milk, which is unappealing. However, butter, ghee, and cream made from buffalo is highly preferred in India, and from sheep in Arab countries. Buffalo milk, with its higher fat percentage and larger fat globules, can give a cream of 56% fat and 5.3% solids not fat (SNF) compared with 50% fat and 3.1% SNF for cow milk (Park & Haenlein, 2006). Buffalo butter is preserved by the addition of 0.2% ascorbic acid and by decreasing the cream pH to 6 and increasing the rate of acid development, but not in cow butter; the addition of salt can also inhibit lipolysis in butter prepared from ripened buffalo cream, which can be stored up to 90 days at low temperatures. Worldwide, cow milk dairy products are preferred, followed by other milks (Park & Haenlein, 2006).

18.3 NUTRITIVE VALUES OF BUTTER, GHEE, AND CREAM

Dairy products, such as butter, ghee, and cream, have been considered as a basic nutrient-dense food that can deliver many energy-rich nutrients. These energy-rich nutrients include a large variety of essential nutrients like fats, minerals, vitamins, and amino acids, and are important to support overall body function, along with various health beneficial compounds, such as fatty acids, phospholipids, and probiotics, which deliver various functional ingredients. Even though dairy products are recommended for a healthy diet, they are often eliminated due to the presence of saturated fat. However, each dairy product varies in composition and is discussed in relation to their beneficial health properties.

18.3.1 Butter

The composition of butter from bovine milk is listed in Table 18.1. Proximate compositions include fat 81.1%, carbohydrate 0.1%, protein 0.9%, ash 2.1%, and water 15.9%. Fat compositions are listed in Table 18.2. Butter has a saturated fat content around 51.4%, with saturated fats, such as palmitic and stearic acid, which are double the concentration of those in cream and other dairy products. However, stearic acid is beneficial to health and lowers the level of low-density lipoprotein (LDL) in blood. Butyric acid is at a greater concentration in butter than other short-chain fatty acids (SCFAs). It causes a rancid off-flavor in

Table 18.1. Proximate composition of butter, ghee, and cream products (%) from cow milk.

Constituents	Butter	Ghee	Cream
Fat	81.11	99	37.00
Carbohydrate	0.06	—	2.79
Protein	0.85	0.29	2.05
Ash	2.11	—	0.45
Water	15.87	0.27	57.71

Source: adapted from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

Table 18.2. Fat composition in butter, ghee, and cream products (g per 100 g) from cow milk.

Fat	Common name	Systematic name	Butter*	Ghee	Cream
Saturated fatty acids			51.37	57.46	23.03
4:0	Butyric	Butanoic	3.226	2.994	1.20
6:0	Caproic	Hexanoic	2.007	1.769	0.710
8:0	Caprylic	Octanoic	1.190	1.030	0.413
10:0	Capric	Decanoic	2.529	2.313	0.928
12:0	Lauric	Dodecanoic	2.587	2.596	1.039
14:0	Myristic	Tetradecanoic	7.436	9.287	3.721
16:0	Palmitic	Hexadecanoic	21.697	24.280	9.732
18:0	Stearic	Octadecanoic	9.999	11.187	4.484
20:0	Arachidic	Icosanoic	0.138	—	—
Monounsaturated fatty acids			21.021	26.666	10.686
16:1	Palmitoleic	*cis*-9-Hexadecenoic	0.961	2.066	0.829
18:1	Oleic	*cis*-9-Octadecenoic	19.961	23.222	9.308
Polyunsaturated fatty acids			3.043	3.429	1.374
18:2	Linoleic	*cis*, *cis*-9,12-Octadecadienoic	2.728	2.088	0.836
18:3	Linolenic	*cis*, *cis*, *cis*-9,12,15-Octadecatrienoic	0.315	1.341	0.538
Cholesterol			0.215	0.300	0.137
Phospholipids			0.1–0.25	0.010	0.1–0.5

*The proportion of fat in cream is 37%.

Source: adapted from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

butter, which is undesirable during storage. However, phospholipids have many biological activities which are essential for human health.

Amino acids, minerals, and vitamins in butter are listed in Tables 18.3 and 18.4. Glutamic acid, leucine, and proline are a little more common than other amino acids in cream. Minerals are in lower concentrations in butter than in cream. Major minerals, such as calcium, magnesium, and potassium, are a little higher than other minerals. Fat-soluble vitamins are a little higher in butter than in cream, such as vitamin A, carotene, and vitamin K. Vitamin K has a protective role in hepatocarcinoma.

18.3.2 Ghee

The composition of ghee based on cow milk is listed in Table 18.1. Proximate compositions of ghee are fat 99%, protein 0.3%, and water 0.3%. Fat compositions are listed in Table 18.2. Saturated fatty acids (57.5 g per 100 g) are the major component of fat in ghee. Predominant saturated fatty acids include palmitic, stearic, and myristic acid. The concentrations of these fatty acids are higher than found in butter. However, SCFAs in ghee, such as butyric, caproic, caprylic, and capric acid, are in lower concentrations than in butter. This give ghee a longer storage life than butter with less rancid off-flavor. These fatty acids are potentially beneficial for reducing body weight and body fat. Further, these fatty acids are easily digestible and transferred directly from the intestine to the portal circulation and are a preferred source of energy (β-oxidation). Higher concentrations of monounsaturated and polyunsaturated fatty acid are found in ghee, and these have various biological benefits.

Amino acids, minerals, and vitamins in ghee are listed in Tables 18.3 and 18.4. Total amino acid concentrations are a little lower in ghee than in butter. Minerals are relatively low in ghee. Fat-soluble vitamins, such as vitamin A, carotene, and vitamin K, are found in similar concentrations to those in butter. The heating process of ghee causes certain losses in vitamins, and in particular water-soluble vitamins are negligible in ghee (Table 18.4).

18.3.3 Cream

The composition of cream based on bovine milk is listed in Table 18.1. Fat content is about 37%, carbohydrate 2.8%, protein 2.1%, ash 0.5%, and water 57.7%. The major fat compositions are listed in Table 18.2. Saturated fatty acids are about 23.0 g per 100 g, including palmitic, stearic, myristic, and lauric acid. Palmitic acid is one of the major saturated fatty acids; it raises serum cholesterol while stearic acid does not (Grundy, 1994). The low

Table 18.3. Amino acids in butter, ghee, and cream products (g per 100 g) from cow milk.

Amino acids	Butter	Ghee	Cream*
Tryptophan†	0.012	0.007	0.029
Threonine†	0.038	0.015	0.093
Isoleucine†	0.051	0.015	0.124
Leucine†	0.083	0.022	0.201
Lysine†	0.067	0.022	0.163
Methionine†	0.021	0.007	0.051
Cystine	0.008	0	0.019
Phenylalanine†	0.041	0.015	0.099
Tyrosine	0.041	0.015	0.099
Valine†	0.057	0.015	0.137
Arginine	0.031	0.007	0.074
Histidine†	0.023	0.007	0.056
Alanine	0.029	0.007	0.071
Aspartic acid	0.064	0.022	0.156
Glutamic acid	0.178	0.058	0.429
Glycine	0.018	0.007	0.043
Proline	0.082	0.022	0.199
Serine	0.046	0.015	0.111
Total	0.890	0.278	2.154

*The proportion of fat in cream is 37%.

†Essential amino acids.

Source: based on data from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

concentration of butyric acid can inhibit a wide range of human cancers, such as colorectal cancer (Parodi, 1997, 2005). However, an increasing concentration of butyric acid is undesirable in cream because of its rancid off-flavor. Monounsaturated and polyunsaturated fatty acids in cream show various health benefits. Oleic acid is at relatively higher concentration than the other polyunsaturated fatty acids, and acts as a great energy source. Jones *et al*. (2008) showed that the oxidation rate of oleic acid exceeded that of linoleic acid in healthy adult male subjects. In contrast, some researchers investigated isotope-labeled fatty acids and reported that of the 18-carbon fatty acids, linolenic acid is the most highly oxidized, followed by oleic acid, and then linoleic acid. Linolenic acid also benefits humans through its various bioactive properties, such as prevention of cancer, prevention of hypertension, and improvement of vision. Cholesterol in cream has a negative impact on all types of cream. Phospholipids have low concentrations in cream. However, these compounds exhibit several health beneficial functions, including antioxidative and antitumor properties in the human body.

Table 18.4. Mineral and vitamin constituents in butter, ghee, and cream products (per 100 g) from cow milk.

Compound	Butter*	Ghee†	Cream*‡
Minerals			
Ca (mg)	24.00	7.25	65.00
P (mg)	24.00	0	62.00
K (mg)	24.00	7.25	75.00
Na (mg)	11.00	0	38.00
Mg (mg)	2.00	0	7.00
Zn (mg)	0.09	0	0.23
Mn (mg)	0	0	0.001
Fe (mg)	0.02	0	0.03
Cu (mg)	0	0	0.006
F (μg)	2.80	–	3.00
Se (μg)	1.00	0	0.50
Vitamins			
Vitamin A (IU)	2499	2849	1470
Carotene (μg)	193	181	72.00
Thiamin (mg)	0.005	0	0.022
Riboflavin (mg)	0.034	0.007	0.110
Pyridoxine (mg)	0.003	0	0.026
Nicotinic acid (mg)	0.042	0	0.039
Cobalamin (μg)	0.170	0	0.18
Folic acid (μg)	3.00	0	4.00
Pantothenate (mg)	0.110	0.001	0.255
Ascorbic acid (mg)	0.00	0	0.60
Vitamin D (μg)	0.90	0	1.40
Tocopherol (mg)	2.32	2.61	1.06
Vitamin K (μg)	7.00	7.98	3.20

*Based on data from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

†Based on data from http://www.indiacurry.com/nprofiles/butteroilgheenutrition.htm

‡The proportion of fat in cream is 37%.

Amino acids, minerals, and vitamins in cream are listed in Tables 18.3 and 18.4. They are minor constituents in cream, but have various health benefits. Among the amino acids, glutamic acid, leucine, proline, aspartic acid, lysine, and valine occur in higher quantities in cream and are essential for various biological functions in human health. In addition, tyrosine, histidine, and arginine are semi-essential amino acids for children, because the metabolic pathways that synthesize these amino acids are not fully developed. Minerals, such as potassium, calcium, and magnesium, are common in creams, and are essential for bone health. Recommended daily intake of calcium is

about 800 mg/day for an adult. However, daily intake of calcium varies in people and in general old people require more calcium than younger people to prevent osteoporosis. The iron content of cream (0.03 mg per 100 g) is insufficient since the daily requirement of iron is about 10 mg/day for men, 12 mg/day for women and 20 mg/day for children. Recent approaches to microencapsulation of iron can overcome iron deficiency and development of rancid off-flavor if dairy products are fortified (Kwak *et al*., 2003). Vitamins, such as vitamin A, carotene, folic acid, vitamin D, and vitamin K, are found in cream. Vitamins A, D, E, K, and carotene are highly lipophilic and are derived from their milk sources. These vitamins have high nutritional value for the newborn. Some vitamins are highly bioactive, for example vitamin A plays an important role in morphogenesis and carotenoids play an effective role in fertility. Water-soluble vitamins, such as folate and vitamin B_{12}, play an important role in reducing the effects of Alzheimer disease in old people.

Overall, butter, ghee, and cream are fat-enriched products which have higher content of fat and essential fatty acids, such as linoleic acid, than of protein, carbohydrate, and minerals. Butter, ghee, and cream made from other milks, such as goat, has certain nutritional advantages because of the greater availability of medium-chain fatty acids (MCFAs) and SCFAs (Park & Haenlein, 2006). These contribute to human nutrition in many ways: they assist digestion; they have a hypocholesterolemic effect; and are also used therapeutically, for example for gallstones, cystic fibrosis, and coronary bypass. Buffalo cream, butter, and ghee are predominantly used in the Indian subcontinent, and are rich in saturated fat; however, total cholesterol content is found to be lower in buffalo ghee, with increased butyrate content in buffalo ghee (Park & Haenlein, 2006). In Russia and other Asian countries, mare milk products have been used for centuries in the treatment of various diseases and are very beneficial to human health. Small quantities of these dairy products are also made from other milks, such as yak, reindeer, camel, but the nutritional significance is yet to be studied. However, these products are rich in saturated fats, which should be taken in consideration before consumption.

18.4 HUMAN HEALTH BENEFIT COMPONENTS IN BUTTER, GHEE, AND CREAM

Butter, ghee, and cream products play an important role in supplying various health-enhancing components to the human diet. These components are found mainly in MFGM, CLA, and SCFAs. They aid in the prevention of various diseases, such as osteoporosis, cancer, atherosclerosis, and other degenerative disorders in humans. Some components are endowed with nutrients, such as peptides, lipids, minerals, and vitamins, which have bioactive properties along with beneficial effects, and they extend the lifespan of humans. During the manufacture of sour cream and butter, lactic acid bacteria are added, which can generate various metabolites during the fermentation process. These probiotic microorganisms exert their beneficial properties through two mechanisms, indirectly through supplementing metabolites and directly by providing live cells. The sphingolipids and their metabolites have health-enhancing functions, including antimicrobial activity on certain pathogens like *Listeria monocytogenes*, inhibition of colon cancer, and regulation of the immune system. Dahi, a fat-enriched Indian sour cream product made from milk, has various health beneficial activities because of the rich supply of lactic acid bacteria and their metabolites produced during the fermentation (Vijayendra *et al*., 2008).

18.4.1 Milk fat globule membrane

MFGM is a highly structured membrane that surrounds the milk fat globules and contains unique beneficial lipids and specific proteins. The primary lipids of MFGM are polar lipids and significant amounts of neutral lipids, such as cholesterol, triglycerides, diglycerides and monoglycerides (Wooding & Kemp, 1975). Isolation methods have identified the content of the MFGM neutral lipids, particularly triglycerides (Walstra, 1974, 1985; Kwak *et al*., 1989). Health attributes of MFGM mainly involve the polar lipids, such as sphingomyelin, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl choline, phosphatidyl serine, glucosyleramide, lactosyl ceramide and gangliosides (Deeth, 1997; Danthine *et al*., 2000). In addition, some MFGM proteins are found to have health benefits, for example fatty acid binding protein, xanthine dehydrogenase/oxidase (XDH/XO), butyrophilin, BRCA2, and BRCA1.

18.4.2 Health benefits of MFGM polar lipids

MFGM polar lipid fractions consist mainly of sphingolipids and glycerophospholipids. Sphingolipids are functional ingredients because of the presence of health beneficial components, such as sphingomyelin and its metabolites including sphingosine and ceramide (Schmelz *et al*., 2000). The metabolites of sphingolipids serve as second messengers that play a vital role in various cell activities, such as regulation, proliferation, and growth (Futerman & Hannun, 2004). Some metabolites have an opposite function in the cell: sphingosine and ceramide are antimitogenic and inhibit cell growth (Sweeney *et al*., 1998) while sphingosine 1-phosphate (S1P) is mitogenic. Defects in serine palmitoyl transferase suggest that exogenous feeding of sphingolipids is necessary for cell growth. Sphingomyelin is sequentially hydrolyzed by various intestinal enzymes and results in the synthesis of ceramide and sphingosine. These metabolites are easily absorbed by the intestine.

Small amounts of ingested metabolites are excreted in feces (Nilsson, 1969). Some studies report that feeding rats with sphingomyelin benefited neonatal gut maturation during suckling (Oshida *et al*., 2003).

18.4.3 Sphingolipids: anticholesterol effect and heart disease

In the group of sphingolipids, sphingomyelin was found to minimize the intestinal uptake of cholesterol and other fats in rats (Noh & Koo, 2003). Pharmacological inhibition in the metabolism of sphingolipids can lead to obesity and cardiovascular diseases. The inhibitory effect was found to be greater with milk-derived sphingomyelin than egg-derived sphingomyelin by the direct inhibitory effect of the sphingomyelin long-chain fatty acyl group on lipolysis in the rumen (Noh & Koo, 2004; Spitsberg, 2005). Interaction was favored by saturation of the sphingomyelin long-chain fatty acyl chain (Eckhardt *et al*., 2002). Dietary uptake of sphingolipids also plays an important role in lowering plasma triacylglycerol and cholesterol (Duivenvoorden *et al*., 2006). It helps in preventing cardiovascular disease, deposition of fat in liver, and other inflammatory disease. Sphingolipid metabolites, such as ceramide and ganglioside GM3, are also assumed to play a major role in the process. However, over-uptake may also lead to the risk of certain metabolic disorders (Parillo & Riccardi, 2004). Lysosphingolipids present in high-density lipoprotein (HDL) are also found to be beneficial for the heart (Podrez, 2010). It protects the heart by releasing nitric oxide (Nofer *et al*., 2004). In a different study, a positive correlation was observed between neutral and acid sphingomyelinase activity and atherosclerosis (Pavoine & Pecker, 2009).

18.4.4 Sphingolipids and cancer

Sphingolipid levels and the enzymes involved in metabolizing sphingolipids are found to be altered in cancer (Ryland *et al*., 2011). Dietary intake has positive effects on the progression of cancer; however, the mechanism is not clear. The rapid turnover of intestinal cells is delayed in cancer, and sphingomyelin showed benefits through ceramide and sphingosine by inducing cell differentiation and apoptosis (Merrill *et al*., 2001). Inhibitory levels of sphingolipids were found at both stages of colon tumorigenesis in mice, and also in the shift from malignant to benign in adenocarcinomas. A decrease in activity of sphingomyelinase may limit the production of certain metabolites that may have anticancer effects on colon cells. Even though sphingolipids have been found to have anticancer properties, human trials have not yet confirmed this. Some sphingomyelin metabolites, such as ceramide, were found to have antitumor effects, whereas sphingosine was found to be mutagenic through its metabolites (Zhang *et al*., 1990). However, some concentrations of sphingolipids had detectable effects in mice and humans (Vesper *et al*., 1999).

18.4.5 Sphingolipids: bactericidal effect

Sphingolipids have been found to be protective against certain types of bacteria, viruses, and certain toxins through competitive inhibitory mechanisms. Among the sphingolipids, glycosphingolipids act as a membrane receptor that induces signaling and mediates infection via the membrane (Kaida & Kusunoki, 2010). Supplementing the diet with sphingolipids may prevent bacterial adhesion and shift the microbial load to the colon. Supplementation of certain sphingolipids in infant diets was found to increase the level of pathogenic bacteria in feces (Sprong *et al*., 2001). However, certain sphingolipid metabolized products, such as ceramide, were found not to be bactericidal, whereas lysosphingomyelin had greater bactericidal effects against *L. monocytogenes* (Sprong *et al*., 2001).

18.4.6 Sphingolipids: effects on diabetes mellitus and Alzheimer disease

Sphingolipid metabolites serve as promoters and inhibitors for diabetes mellitus. Both types of diabetes mellitus occur because of reduced β-cell mass, which ultimately leads to decreased proliferation and increased apoptosis of liver cells (Hui *et al*., 2004). It also leads to insufficient amounts of insulin. The different metabolites of sphingolipids serve as regulators of β-cell survival, proliferation, and function. Oversupply of nutrients, in particular fatty acids, may lead to metabolic disorders (Parillo & Riccardi, 2004). Metabolites, such as ceramide, produced due to excessive deposition of saturated fat, can inhibit the production of insulin by inducing β-cell apoptosis (Kelpe *et al*., 2003; Maedler *et al*., 2003). Some ceramide glycosylated derivatives, such as gangliosides, have been found to be antigens in certain autoimmune diseases (Misasi *et al*., 1997). Certain glycosphingolipid derivatives have a vital role in Alzheimer disease. The conformational change of amyloid β-protein from coiled to the more ordered β-sheet is facilitated by binding gangliosides. Age-related diseases are also associated with sphingolipids. In most tissues, changes in the content of sphingomyelin may lead to aging. Metabolized products, such as ceramide, act as senescence mediators in aging cell culture models (Vesper *et al*., 1999).

18.4.7 Sphingolipids and multiple sclerosis

Multiple sclerosis affects more women than men and there are an estimated 400 000 patients in the USA (Compston & Coles, 2008). This chronic demyelinating disease is characterized by infiltrates in the central nervous system that lead to disability. Sphingolipids and their metabolites play

an important role in the disease (Walter & Fassbender, 2010). They act as mediators of S1P, which binds to receptors of S1P1 and S1P4 on lymphocytes. The levels of expression on lymphocytes are varied: higher amounts are found in the lymph nodes and lower levels in the bloodstream (Lo *et al*., 2005).

18.4.8 Phospholipids

Phospholipids are essential fatty acids and are essential for all living cell membranes, especially brain cells. They are bipolar in structure, which is essential for the biological functions of the cell membrane and provides stability. They comprise phosphatidyl choline (lecithin), phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidyl inositol. A few recent studies have reported the health benefits of MFGM phospholipids in lipid metabolism. Wat *et al*. (2009) reported that diets rich in phospholipids from dairy extracts reduced lipid levels in mice on a high-fat diet. A similar study reported that in mice the accumulation of cholesterol in hepatic cells was reduced after feeding them milk rich in phospholipids, with a significant increase in fecal cholesterol (Kamili *et al*., 2010). Health benefits vary with the concentration of phospholipids; phosphatidyl serine had limited significance since the concentration is very low in dairy products (Rombaut & Dewettinck, 2006). Supplementation of phosphatidyl serine in exercising humans altered endocrine function and well-being. Supplementation with 200 mg/day of phosphatidyl serine showed improvements in patients with Alzheimer disease (Heiss *et al*., 1994; Hashioka *et al*., 2004).

Milk phospholipids play an important protective role in the duodenal mucosa of humans (Kivinen *et al*., 1992). Digested phospholipids, such as lysophosphatidyl choline, have a greater protective role against bacteria (van Rensburg *et al*., 1992). However, some lysophosphoglycerides have moderate sensitivity to Gram-positive bacteria and are not sensitive to Gram-negative bacteria (Sprong *et al*., 2001). Toxic and chemical attacks are prevented by phosphatidyl choline, which leads to less damage to the liver (Kidd, 2002). In infants, life-threatening toxic attacks on the gastrointestinal mucosa were prevented by phosphatidyl choline (Carlson *et al*., 1998). It also acts as a good source of choline, which helps in the synthesis and transport of neurotransmitters that aid in development of the brain (Blusztajn, 1998). Furthermore, gastrointestinal digested phospholipid compounds also exhibit antimicrobial activity (van Hooijdonk *et al*., 2000).

18.4.9 Protein fractions of MFGM

The protein fractions of MFGM occur in a 1 : 1 weight ratio (Kanno & Kim 1990), with polypeptides in the range 10–300 kDa (Mather, 2000). Most of MFGM proteins are glycoproteins; the major protein is butyrophilin (40%), followed by XO (12–13%), and other minor proteins (<5%) (Mather, 2000; Spitsberg, 2005). Some MFGM proteins show health benefits.

18.4.9.1 Anticancer effects

Various research reports suggest that MFGM proteins have a preventive role in cancer cell growth in humans (Spitsberg *et al*., 1995; Spitsberg & Gorewit, 1997, 2002; Peterson *et al*., 1998). Among MFGM proteins, fatty acid binding protein at a very low concentration inhibits the growth of breast cancer cell lines (Kromminga *et al*., 1990). In addition, BRCA1 and BRCA2 proteins are involved in the DNA repair process (Spitsberg & Gorewit, 1998). Colon cancer develops mainly due to the toxicity of degraded glucuronides produced by the intestinal bacterial enzyme β-glucuronidase. Dietary supplementation of MFGM can prevent colon cancer with the aid of β-glucuronidase inhibitor. In *in vitro* studies, MFGM proteins inhibited *Escherichia coli* β-glucuronidase (Ito *et al*., 1993). Spitsberg hypothesized that dietary supplementation with MFGM released inhibitory peptides after digestion in the digestive tract, which could enter the bloodstream and exhibit inhibitory action on cells of tissues or organs undergoing carcinogenic transformation (Spitsberg *et al*., 1995; Spitsberg, 2005).

18.4.9.2 MFGM proteins, autism, and multiple sclerosis

The causes and etiology of autism and multiple sclerosis are unknown, but environmental and genetic conditions are possible factors. The autistic brain has structural abnormalities that are similar to the neurodevelopmental disorder (Purcell *et al*., 2001; Vojdani *et al*., 2002). Some researchers have proposed that butyrophilin can reduce the development of autistic behavior and modulate the autoimmune response. Multiple sclerosis is considered to be a neurodegenerative disease that affects the central nervous system (Lauer, 1997). Supplementation with butyrophilin suppressed progression of encephalomyelitis, a disease which shows similar characteristics to human multiple sclerosis (Mana *et al*., 2004).

18.4.9.3 Antibacterial and antiadhesive effects of MFGM proteins

MFGM proteins act as good antibacterial and antiadhesive agents in the gastrointestinal tract of humans. Among the MFGM proteins, XO accounts for about 13%, which serves as a good antimicrobial agent by producing reactive oxygen, hydrogen peroxide, and peroxynitrite, and by reducing inorganic nitrite to nitric oxide (Harrison, 2006). MFGM protein receptors can bind to pathogenic bacteria

and thereby prevent the binding of epithelial membranes in the digestive tract. XO can also inhibit the growth of certain bacteria, such as *E. coli* and *Staphylococcus aureus*, by formation of hydrogen peroxide (Martin *et al*., 2004). Another protein, lactophoricin, also shows inhibitory activity against Gram-positive and Gram-negative bacteria. A possible mechanism is due to pore forming capacity. Stomach diseases, such as peptic ulcer and stomach cancer, occur because of colonization of stomach mucosa with *Helicobacter pylori*, that leads to hemagglutination (Fox & Wang, 2007). MFGM delipidated mucins show similar inhibitory action of gastric mucins. However, the low molecular weight protein glycomacropeptide shows less activity than mucins.

In a study of mice, bovine milk glycoprotein significantly reduced infection and gastric colonization by *H. pylori*. Both MFGM and defatted MFGM showed similar healing rates in *H. pylori*-infected BALB/cA mice (Wang *et al*., 2001). The slightly different structure of bovine MFGM protein can vary the preventive role of infection compared with the human protein counterpart. Human lactadherin can inhibit a rotavirus whereas MFGM lactadherin does not. MUC1 protein also inhibits the colonial growth of *E. coli* (Peterson *et al*., 1998).

18.5 CONJUGATED LINOLEIC ACID

After being identified as a cancer-suppressing agent, CLA has attracted a lot of attention with regard to its functional effects on preventing diabetes, obesity, atherosclerosis, and many other health problems, gaining a reputation as a panacea (Benjamin & Spener, 2009). CLA is group of *trans*-fatty acids and represents the positional and geometric isomers of octadecadienoic acid derived from linoleic acid. These bioactive components are predominantly found in milk and milk products, along with ruminant meat products (Steinhart *et al*., 2003). A high concentration of CLA is found in milk fat: butter and ghee are particularly renowned as the richest sources of CLA; CLA content in ghee was found to be as high as 600 mg per 100 g, while 300 mg was found in butter; the CLA content of cream has yet to be defined (Parodi, 1994; Sserunjogi *et al*., 1998). Among the large number of CLA isomers isolated from milk fat, *cis*-9, *trans*-11 comprises approximately 90% while less than 10% is represented by *trans*-10, *cis*-12; health beneficial functions are associated with these two major isomers (Bhattacharya *et al*., 2006). On the basis of recent research findings, CLA has anticarcinogenic, antidiabetic, antiobesity, antiatherogenic, osteosynthetic, and immunomodulatory effects. Even though these effects are for animal models with very few human studies, there are prospective implications in human subjects with suitable dose and isomer.

18.5.1 Carcinogenesis

Since Pariza's group first determined the anticarcinogenic effects of CLA, it has drawn remarkable attention as an anticarcinogen (Ha *et al*., 1987). Later research outcomes revealed that CLA reduced the risk of various types of cancers, including gastric, colorectal, and breast cancers (Table 18.5). CLA has also been examined for chemopreventive functions in inhibiting, retarding, or reversing multiple types of cancers. To establish the inhibitory effects of CLA at different stages of cancer development, a number of animal models have been used, but studies with human subjects are rare (Lee & Lee, 2005). Although a mixture of CLA isomers have been proven to suppress numerous types of cancers, recently individual CLA isomers have been demonstrated to have distinct effects on cancer prevention (Belury, 2002; Lee & Lee, 2005; Bhattacharya *et al*., 2006). Of the two physiologically important isomers, although *cis*-9, *trans*-11 CLA predominates over *trans*-10, *cis*-12 CLA in ruminant meat and dairy products, the latter outperforms the other in terms of anticancer effects (Kelley *et al*., 2007).

18.5.2 Colonic and colorectal cancer

Cancers of the colon and rectum are among the most commonly diagnosed diseases in the USA, and every year a large number of deaths are attributable to these cancers (Miller *et al*., 2008). CLA has been extensively studied as a chemopreventive factor in cancer of the colon. Kim *et al*. (2002) provided the first evidence that the *trans*-10, *cis*-12 isomer inhibits the growth of the human colon adenocarcinoma cell line Caco-2. This report illustrated that some insulin-like growth factors (IGFs) stimulated Caco-2 cell proliferation and tumorigenesis, and were successfully downregulated by the *trans*-10, *cis*-12 isomer. Several elements of IGFs play important roles in the development of colon cancer (Singh & Rubin, 1993). A cell culture study by Cho *et al*. (2003) stated that the *trans*-10, *cis*-12 isomer decreased HT-29, a human colon cancer cell line; these authors suggested that the reduction in HT-29 cell number by the *trans*-10, *cis*-12 isomer may at least in part be mediated by decreasing secretion of IGF-II. CLA can also suppress intestinal inflammation and prevent colonic carcinogenesis by activating the nuclear hormone receptor PPARγ (Evans *et al*., 2010).

18.5.3 Breast cancer

Cumulative evidence suggests that CLA isomers retard the proliferation of human mammary tumor cells. Treating breast cancer MCF-7 cells containing wild-type p53 with CLA inhibited cell proliferation. Mechanistically, cell cycle arrest by CLA occurred via two actions: CLA

Table 18.5. Anticarcinogenic and antidiabetic effects of conjugated linoleic acids in butter, ghee, and cream products for health benefits.

Isomer	Function	Reference
Carcinogenesis		
trans-10, *cis*-12	Inhibition of proliferation of MCF-7 breast cancer cells	Kemp *et al*. (2003)
trans-9, *cis*-11	Suppression of caveolin-1 expression in MCF-7 breast cancer cells	Huot *et al*. (2010)
trans-10, *cis*-12	Reduction of Caco-2 colon cancer cells and gene expression	Kim *et al*. (2002)
cis-9, *trans*-11; *trans*-10, *cis*-12	Inhibition of colorectal cancer	Palombo *et al*. (2002)
trans-10, *cis*-12	Inhibition of proliferation of HT-29 colon cancer cell line	Cho *et al*. (2003)
cis-9, *trans*-11	Suppression of growth and proliferation of SGC-7901 in gastric carcinoma	Liu *et al*. (2002)
Unknown	Suppression of DNA synthesis and increased apoptosis in bladder cancer cells	Oh *et al*. (2003)
Unknown	Suppression of colonic TNF-α mRNA expression	Evans *et al*. (2010)
Diabetes		
trans-10, *cis*-12	Improve insulin resistance	
trans-10, *cis*-12	Improve liver carbohydrate and lipid metabolism	Jourdan *et al*. (2009)
M*	Improves glucose tolerance, increases glucose transport and glycogen synthase activity, and upregulation of UCP-2	Ryder *et al*. (2001)
Unknown	Increases PPARγ in adipose tissue and improves insulin resistance	Zhou *et al*. (2008)
Unknown	Enhances plasma adiponectin level	Nagao *et al*. (2003)
M	Maintains insulin sensitivity	Parra *et al*. (2010)
trans-10, *cis*-12	Decreases blood glucose, plasma leptin, body weight, and body mass index	Belury *et al*. (2002)

M* is a 50 : 50 mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12.

induced the accumulation of the tumor suppressor proteins p53, p27, and p21, while expression factors for cell cycle progression from G_1 to S phase were suppressed by CLA (Kemp *et al*., 2003). Another potential mechanism by which CLA suppresses MCF-7 breast cancer cell replication is a consequence of altering the lipid microenvironment of caveolae and expression of caveolae-resident protein by CLA (Huot *et al*., 2010). Caveolae are special types of membrane structure which are supposed to affect many facets of cancer cell function, including growth, cell signaling, and apoptosis. The antitumor activity of CLA on breast cancer cells is also ascribable to its antiestrogenic properties in the affected tissue (Tanmahasamut *et al*., 2004).

18.5.4 Gastrointestinal cancer

The chemoprotective roles of CLA against gastrointestinal cancer have been studied lately, demonstrating that CLA can modulate both apoptosis and metastasis in cancerous cells in the gastrointestinal tract. Apoptosis, one of the important cellular events, is regarded as programmed cell death. This consequence is desirable for cells affected with cancer, and it has been demonstrated that *cis*-9, *trans*-11 CLA suppresses the proliferation of SGC-7901 human gastric cancer cells by inducing apoptosis (Liu *et al*., 2002). Antimetastatic functions of CLA were brought to light when Kuniyasu *et al*. (2006) reported that, besides antiproliferative effects, CLA could decrease epidermal growth factor receptor production and transforming growth factor (TNF)-α secretion in MKN28 human gastric cancer cells and Colo320 human colon cancer cells. This study associated the antimetastatic effect of CLA with its capability to induce PPARγ activity.

The discovery of abundant quantities of CLA in milk and dairy products and its anticarcinogenic potential has prompted researchers from different fields to examine extensively the anticancer effects of CLA. Besides the types of cancers mentioned earlier, CLA has also been demonstrated to have functional effects on skin and bladder cancers (Oh *et al*., 2003; Belury *et al*., 2007). Despite the fact that CLA's potential to be used in cancer

chemotherapy is tempting, extrapolation of the available findings to human therapy is still premature unless adequate clinical trials are carried out.

18.5.5 Diabetes

With the ever-increasing incidence of type 2 diabetes, there is an urgent need to find a suitable therapeutic agent. As a possible medication, CLA has been studied in a number of animal models and human subjects, with the implication that CLA is capable of decreasing blood glucose levels and improving insulin resistance in humans (Benjamin & Spener, 2009). It is also postulated that CLA can improve glucose tolerance and insulin-stimulated glucose transport; CLA has been shown to ameliorate liver carbohydrate and lipid metabolism in insulin-deficient mice (Ryder *et al*., 2001; Jourdan *et al*., 2009) (Table 18.5).

The effects of CLA on glucose metabolism are presumably collateral events mediated either by enhancement of PPARγ activity or by upregulation of expression genes like uncoupling proteins (Ryder *et al*., 2001; Syvertsen *et al*., 2007). Another mode of action by which CLA maintains insulin sensitivity is the activation of adiponectin, a recently discovered hormone which has been reported to enhance insulin activity (Nagao *et al*., 2003; Parra *et al*., 2010). However, human and animal studies have recently given conflicting results: regardless of some antidiabetic functions, the *trans*-10, *cis*-12 isomer might induce liver steatosis and insulin resistance (Moloney *et al*., 2004; Halade *et al*., 2010).

18.5.6 Obesity

Following the discovery by Park *et al*. (1997) that CLA modulates body composition properties, a great deal of interest has been directed toward identifying the antiobesity characteristics of CLA in order to combat the prevalence of obesity. Some researchers have demonstrated that CLA consistently reduced body fat (Sisk *et al*., 2001; Evans *et al*., 2002; Hargrave *et al*., 2002; Nagao *et al*., 2003; Yamasaki *et al*., 2003) (Table 18.6). Of the two major isomers, *trans*-10, *cis*-12 CLA is believed to be responsible for antiobesity effects (Kennedy *et al*., 2010). A number of mechanisms have been proposed to explain the antiobesity effects of CLA, such as regulation of energy metabolism, modulation of adipokines and cytokines, and increasing fatty acid β-oxidation.

CLA helps in enhancing energy metabolism by increasing energy expenditure, which is indicated by increased oxygen consumption (Nagao *et al*., 2003; Choi *et al*., 2004). In addition, CLA reduces body fat mass, supposedly by inhibiting lipoprotein lipase levels in adipose cells (Lin *et al*., 2001), suppressing stearoyl-CoA desaturase activities (Park *et al*., 2000), and enhancing apoptosis of pre-adipocytes and adipocytes (Brown *et al*., 2003, 2004). Finally, CLA is supposed to reduce body fat by modulating adipokines and cytokines. It has been shown that CLA may reduce the secretion and expression of leptin; this is explained by the fact that CLA reduces the total number of adipose tissues (Nagao *et al*., 2003; Park & Pariza, 2007). Some studies have indicated that CLA raises adiponectin with a decreasing level of TNF-α, which is supposed to be one of the key mediators in reducing obesity (Inoue *et al*., 2004; Nagao *et al*., 2005). According to some authors, modification of proinflammatory cytokines, such as interleukin, by CLA is designated as an important mechanism behind its antiobesity functions (Bassaganya-Riera *et al*., 2003; Changhua *et al*., 2005).

18.5.7 Atherosclerosis

Atherosclerosis is the accumulation of lipids in inflammatory cells, platelet aggregation, and calcium deposition in medium-sized and large arteries (Toomey *et al*., 2003; Desroches *et al*., 2005). Some animal experiments have shown CLA, as a dietary fatty acid, to be very useful in producing antiatherogenic effects (Kritchevsky *et al*., 2004; Toomey *et al*., 2006; Valeille *et al*., 2006) (Table 18.6). The antiatherogenic effects of CLA are considered to be the result of several actions: decreased atherogenic LDL-cholesterol and increased antiatherogenic HDL-cholesterol, reduced inflammation in blood vessels by subjugating nuclear NF-κB and apolipoprotein A-I activity, and increased cellular cholesterol efflux by facilitating the overexpression of HDL receptors (Benjamin & Spener, 2009). Valleille *et al*. (2006) discovered that *cis*-9, *trans*-11 CLA in milk fat had antiatherogenic effects in hyperlipidemic hamsters. CLA has also been demonstrated to suppress the migratory and inflammatory phenotype of macrophage cells. The antiatherogenic effects of CLA are attributed to the *cis*-9, *trans*-11 isomer; hence, feeding practices that improve this isomer in milk fat have been encouraged (Valeille *et al*., 2006).

18.5.8 Immunity

The effect of CLA on immunomodulation has been supported by some recent findings from *in vitro* cell culture studies, *in vivo* animal models, and human subjects. It has been documented that CLA suppresses the release of eicosanoid from human vascular smooth muscle cells by altering immunity soluble factors, such as eicosanoids and cytokines, and facilitating immunoglobulin production (Cheng *et al*., 2003; Ringseis *et al*., 2006). The immunomodulating effect of CLA was corroborated when a study with human subjects revealed that dietary CLA supplementation increased the level of plasma IgA and IgM, and decreased IgE

Table 18.6. Antiobesity and other health beneficial effects of conjugated linoleic acids in butter, ghee, and cream products.

Isomer	Functions	Reference
Obesity		
trans-10, *cis*-12	Attenuates the activity of lipogenic transcription factors and their targets	Obsen *et al*. (2012)
Mixture	Enhances fat oxidation and energy metabolism	Ohnuki *et al*. (2001)
trans-10, *cis*-12	Decreases energy intake	So *et al*. (2009)
Unknown	Upregulation of mitochondrial uncoupling proteins	
trans-10, *cis*-12	Increases energy metabolism	Nagao *et al*. (2003)
trans-10, *cis*-12	Suppresses heparin-releasable lipoprotein lipase (HR-LPL) activity	Lin *et al*. (2001)
trans-10, *cis*-12	Inhibits hepatic stearoyl-CoA desaturase activity	Park *et al*. (2000)
trans-10, *cis*-12	Induces body fat loss and apoptosis	Hargrave *et al*. (2002)
trans-10, *cis*-12	Increases fatty acid oxidation in 3T3-L1 preadipocytes	Evans *et al*. (2002)
Atherosclerosis		
cis-9, *trans*-11; *trans*-10, *cis*-12	Suppress the migratory and inflammatory phenotype of the macrophage	McClelland *et al*. (2010)
M	Induces resolution of atherosclerosis	Toomey *et al*. (2006)
cis-9, *trans*-11; *trans*-10, *cis*-12	Reduce atheromatous lesions	Kritchevsky *et al*. (2004)
cis-9, *trans*-11	Reduces peroxidability index and expression of proinflammatory IL-1β gene	Valeille *et al*. (2006)
Immunity		
cis-9, *trans*-11	Enhances antibody synthesis	Ramirez-Santana *et al*. (2011)
trans-10, *cis*-12	Enhances IgA and IgM production	Yamasaki *et al*. (2003)
cis-9, *trans*-11	Reinforces immune response	Ramirez-Santana *et al*. (2009)
Unknown	Reduces the level of IgE	Sugano *et al*. (1998)
Bone metabolism		
trans-10, *cis*-12	Modulates osteoclastogenesis and bone marrow adiposity	Rahman *et al*. (2011)
M*	Decreases activity of proinflammatory cytokines	Rahman *et al*. (2007)
trans-10, *cis*-12	Reduces mRNA for leptin	Warren *et al*. (2003)
trans-10, *cis*-12	Increases overall ash content	Park *et al*. (2011)
Antioxidant		
trans-10, *cis*-12; *cis*-9, *trans*-11	Produce the expression of antioxidant enzymes	Yukiko *et al*. (2009)
Unknown	Induces glutathione synthesis	Arab *et al*. (2006)

M* is a 50 : 50 mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12.

(Song *et al*., 2005). The same study reported that the proinflammatory cytokines TNF-α and interleukin (IL)-1β were lowered by CLA, while the anti-inflammatory cytokine IL-10 was enhanced. Very recently, one study has reported that CLA could help in developing neonatal immunity by enhancing antibody production (Ramirez-Santana *et al*., 2011). Further extensive research is necessary before CLA can used in functional foods as an immunity enhancer.

18.5.9 Bone health

The prospect that CLA may improve skeletal health has been the subject of extensive research recently. It was found that dietary CLA positively affected bone mineral density in postmenopausal women (Brownbill *et al*., 2005). The increase in bone mineral density with a reduced level of osteoclastogenic proinflammatory cytokines in middle-aged mice indicates that CLA contributes to protection

against age-associated bone loss or osteoporosis (Rahman *et al*., 2006). The antiosteoporotic function of CLA was supported when Rahman *et al*. (2011) reported that the *trans*-10, *cis*-12 isomer modulated osteoclastogenesis and bone marrow adiposity. CLA also has been found to exert a protective function against rheumatoid arthritis by reducing inflammatory cytokines (Hur & Park, 2007). CLA is a promising functional ingredient to prevent age-related bone loss by improving bone mineral density and modulating bone formation.

Nowadays, natural dietary supplements are of great importance in the fight against most chronic diseases. Owing to its great potential in preventing many serious health problems, CLA has been targeted as a prospective dietary supplement. Milk and milk products like cream, butter, and ghee are plentiful sources of CLA. Further, altering the feeding programs of dairy cows may result in greater concentration of CLA in milk and dairy products, and make the consumption of those products worthwhile in terms of producing health beneficial effects.

18.6 SHORT- AND MEDIUM-CHAIN FATTY ACIDS

SCFAs and MCFAs are produced by microbial and milk enzymes during fermentation of cream, butter, and ghee; this leads to the buttery flavor of these products (Kinsella, 1975). In addition to the flavor, it increases richness and creaminess of cream and butter (Balcão & Malcata, 1998). These fatty acids show various health benefits including anticancer, antiobesity and antimicrobial properties. Butryic acid found in various milk products serves as a good source of energy for colonial cells and also acts as a regulator of various genes that are responsible for cell differentiation and death (Hamer *et al*., 2008). Some orally ingested butyrate undergoes hydrolysis by stomach lipase and complete hydrolysis in the small intestine, where it is absorbed into the bloodstream and metabolized in the liver (Parodi, 1997). Ingested milk butyrate was found to be effective against certain tumors (Belobrajdic & McIntosh, 2000). MCFAs found in various dairy products reduce various characteristics of metabolic diseases (Pfeuffer & Schrezenmeir, 2007). Dietary substitution with MCFAs also helps in weight reduction (Dulloo *et al*., 1996). Daily intake of 10 g of medium-chain triglycerides (MCTs) aids in weight reduction and in reduction of hip and waist fat for individuals with body mass index of 23 kg/m^2 or more (Dulloo *et al*., 1996). A mixture of MCT and LCT (long-chain triglyceride) is widely used as a healthy alternative to vegetable oils in Japan (Ogawa *et al*., 2007).

In addition to antitumor and antiobesity activities, MCFAs also show antimicrobial properties against various pathogenic microbes. Bovine whey cream free fatty acids, such as lauric acid and myristoleic acid, are found to inhibit the growth of *Candida albicans in vitro* (Clément *et al*., 2007). In another study (Clément *et al*., 2008), MCFAs and linoleic acid inhibited the growth of fungi like *Aspergillus fumigatus* and *C. albicans*. Some of the milk fatty acids were found to be antiviral and inhibited growth by cell disintegration and leakage (Thormar *et al*., 1987). A similar mechanism was also seen in some human milk-derived lipids, which inhibit the growth of *Chlamydia trachomatis* (Lampe *et al*., 1998). In addition to these fatty acids, some fatty acids obtained from the disintegration of bioactive sphingolipids were found to inhibit the growth of *Salmonella enteritidis* and *Campylobacter jejuni* (Sprong *et al*., 2001, 2002). Among the fatty acids obtained from the disintegration of sphingolipids, capric and lauric acids were highly active against pathogenic microbes (Sprong *et al*., 2002). In past research, a group of Indian scientists reported that ghee from goat milk is rich in SCFAs and MCFAs compared with long-chain fatty acids (Ramesh & Bindal, 1987). These fatty acids are also abundant in other species, such as sheep and mare milk products, which show various therapeutic and nutritional properties. Processing and storage increase the free fatty acid content in goat milk products and heating to 65°C causes lower lipolysis due to inactivation of lipases, whereas cold storage and homogenization increase the overall waxy or goaty flavor of the products and also increase the rancidity (Park & Haenlein, 2006). Free fatty acids in unprocessed goat milk is about 40 μL/mL; cold storage and homogenization increase this to 100–120 μL/mL. Further distribution of C12–C16 fatty acids influences the cleavage rate: C12–C16 are mostly distributed in the *sn*-2 position in goat milk as C12–C16 are uniformly in the *sn*-2 and *sn*-1 positions. Consumption of goat and sheep milk products decreases the level of cholesterol and LDL level in humans due to the higher presence of MCTs (about 36% in goat milk), which decreases the synthesis of endogenous cholesterol. However, the amounts of free fatty acid are found to be lower in buffalo milk and ghee. The individual benefits of ghee fatty acids deserve further study.

18.7 NEW APPROACH ON CHOLESTEROL REMOVAL IN BUTTER, GHEE, AND CREAM

Even though milk fat foods like butter, ghee, and cream are nutritious, some components, such as cholesterol and its oxidation products, have negative effects on consumers. Cholesterol contents are about 137 mg/100 g in 37% milk fat cream, 215 mg/100 g in butter, and 300 mg/100 g in ghee. Most consumers do not want such high cholesterol in their diet because of concerns about coronary heart disease. Therefore, reducing the cholesterol in those products may increase their bioactive value. A unique method to

remove over 90% of cholesterol without defect is by the absorption method with β-cyclodextrin. This method increases cholesterol removal to about 97.8% in regular milk cream, about 90% in whipping cream, and about 93.2% in butter (Kwak *et al*., 2001; Shim *et al*., 2003, 2004). The addition of gamma-linolenic acid in butter increases the product value of butter and an *in vitro* study in the rat showed significantly lower blood cholesterol and triglycerides (Jung *et al*., 2005). Recently, cholesterol removal in cow and buffalo ghees was achieved to about 90% by β-cyclodextrin (Kumar *et al*., 2010). For cream, further research has proved that cross-linking of β-cyclodextrin with adipic acid helps to remove over 90% of cholesterol until the eighth time of reuse (Han *et al*., 2007). The entrapped ingredients are negligible and the residual β-cyclodextrin was a trace amount in cream during cholesterol removal by crosslinked β-cyclodextrin (Ha *et al*., 2010). This means that in the future, butter, ghee, and cream with the cholesterol removed will be great health beneficial foods with no loss of beneficial components.

18.8 CONCLUSION

Butter and cream are fat-enriched products with functional ingredients, and have proven beneficial effects. Ghee has a long history as a healthy food and traditionally has been used as a sacred food on the Indian subcontinent for centuries. The large evidence of scientific proof indicates that various bioactive compounds, such as MFGM, CLA, SCFAs, and MCFAs, in butter, ghee, and cream provide beneficial effects on various human diseases. However, these compounds are also rich in saturated fat, which makes consumers hesitate to consume these products. Recent advances in the development of these products, such as low cholesterol and low fat cream, butter, and ghee, will reduce negative attitudes toward their consumption. In addition to the native functional milk components in fermented butter, ghee, and cream, some bioactive components are also derived during fermentation which have been proven to be health beneficial and aid longevity in all human age groups. More research is needed on cholesterol and saturated fat lowering in butter, ghee, and cream, and on their new bioactive components to benefit human health and well-being.

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19
Condensed and Powdered Milk

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19.1 INTRODUCTION

Most microbial and biochemical changes that alter the quality of food occur in the aqueous phase. Water plays a dual role: (i) as a solvent it ensures the transfer of substrates, growth promoters, biological agents and reaction products, which allows reactions to take place in optimal conditions; and (ii) as a reaction substrate, it is involved in hydrolysis reactions (proteolysis, lipolysis). This dual action requires that water is available, which can be characterised by its water activity (a_w), that is the ratio between the partial pressure of the water vapour of the product and the partial pressure of pure water vapour at the same temperature. Any process that reduces this availability also slows down reaction times.

Water activity can be lowered by the crystallisation of solvent water (freezing) or by the addition of highly hydrophilic solutes that bind water molecules through hydrogen or dipolar interactions (salting, sugaring). It can also be obtained by eliminating the available water (concentration, evaporation and drying); in this case the inhibition generated is removed by dilution or rehydration.

This chapter deals with the properties of food powders obtained through drying. This preservation method only slightly alters the nutritional and organoleptic qualities during dehydration and any pre-treatments are well controlled as regards heat and mass transfer.

Given the high latent heat of vaporisation of water (225 kJ/kg at 100°C), the drying process itself is often preceded by a concentration of the dry matter of the product in order to reduce the energy cost of processing. This preconcentration can be achieved by cross-flow filtration (reverse osmosis for example) or by vacuum evaporation. In reverse osmosis, water is removed without phase change by passing through a membrane under the action of a pressure gradient, which reduces the energy cost of water elimination (10–40 kJ/kg water). However, the efficiency of the process decreases with increasing viscosity and osmotic pressure resulting from a concentration of dry matter (proteins and molecules of low molecular weight, respectively). Thus, it is generally not possible to concentrate the product beyond 25% (w/w) of dry matter. Therefore, in this chapter we only deal with concentration by vacuum evaporation, lactose crystallisation and drying, which are the three main unit operations used in the manufacture of condensed milk and dried products.

19.2 WORLD DAIRY POWDER SITUATION

Growth of the world's milk production slowed by only 0.8% to 703 million tonnes (Mt) in 2009. Cow, buffalo, goat, sheep and camel milk production represent 83.4%, 12.9%, 2.2%, 1.3% and 0.2% of the total world milk production, respectively (International Dairy Federation, 2010).

The geographical breakdown of condensed milk production has changed considerably in the past 30 years. World production, which in the 1980s was dominated by the EU, the USA and the former USSR, is now much more scattered, with significant contributions from the Far East (Malaysia, Thailand, Singapore, China, Australia,

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New Zealand) and South America (Brazil, Peru, Chile, Argentina). The Food and Agriculture Organization estimated world production in 2009 to be around 4.7 Mt. According to the data collected from the IDF National Committees and other respondents, condensed milk production decreased last year in most parts of the world: EU (–3%), China (–8%), Chile (20%), Ukraine (–10%), Canada (–8%), Peru (–8%), Russia (–4%) and USA(–6%) (International Dairy Federation, 2010).

According to the International Dairy Federation (2010), world production of whole milk powder (WMP) is estimated at around 4 Mt. Apart from New Zealand and Argentina, the output of WMP decreased in most parts of the world between 2008 and 2009. Production in Europe has even been decreasing for the last 10 years. The decline was greater last year, because it was more profitable for processors to produce and sell skimmed milk powder (SMP) to intervention industries rather than producing and exporting WMP on the world market. Surprisingly, considering its recent development, the output of WMP by China experienced a huge decrease last year. According to US Department of Agriculture estimates, WMP output (including infant formula) decreased last year from 1.12 to 0.98 Mt. This corresponds to a 13% decline, whereas the human compound annual growth rate (CAGR) was about 12% during the period 2000–2007, just before the melamine crisis in China where the deaths of six infants revealed the sale of milk intentionally contaminated with the nitrogen-containing compound melamine. The melamine crisis gave rise to a general mistrust of domestic WMP by local consumers, which eventually led to a significant decrease in Chinese production. The trend now seems to be reversing. According to the Chinese National Bureau of Statistics, milk powder production (mainly WMP and infant formula) has already increased by 10.6% for the first 6 months of 2010.

According to the International Dairy Federation (2010), world production of SMP is estimated at around 4 Mt and, apart from the USA and Australia, the output of SMP increased last year in most parts of the world. Output in Europe was stimulated by the poor economic situation in the dairy sector, leading the European Commission to open intervention and to purchase butter and SMP; 283 000 t of SMP, corresponding to one-quarter of annual production, were actually withdrawn from the market between March and October. Production was strongly increased in New Zealand due to tremendous growth in export, especially to East and South East Asia.

In terms of whey products and casein, in 2009 surplus milk protein worldwide led to a reduction in casein production in most countries where statistics are available. Output in the EU was estimated at around 115 000 t,i.e. 20 000 t less than in 2008. European processors preferred to sell surplus as SMP to intervention industries (International Dairy Federation, 2010).

Liquid whey production results mainly from the industrial production of cheese, which generates more than 80% of the total whey available, and secondarily from casein output. The major processors of whey are therefore located in Europe, North America, Australia and New Zealand, which correspond to the major cheese production areas. In 2009, the USA produced 490 000 t of whey powder and condensed whey, as well as 190 000 t of whey protein concentrates. The production of whey powder within the EU is estimated at around 1.6 Mt (International Dairy Federation, 2010).

19.3 OVERVIEW OF OPERATIONS

19.3.1 Concentration by evaporation

Concentration by evaporation involves exposing a liquid to temperature and pressure conditions that allow vaporisation of the solvent. This process therefore facilitates concentration of non-volatile elements of the treated product. In the food industry, it is mainly used to remove water from true solutions, emulsions and/or colloidal solutions.

A key aspect of this technique is the energy cost involved, since water is removed by a phase change (liquid–vapour), contrary to separation techniques. Most of the technical developments made were aimed at improving efficiency.

Furthermore, processed food liquids are often heat sensitive. To minimise the biochemical alteration of components, concentration by evaporation is generally carried out under a partial vacuum to reduce the processing temperature by 45°C to 80°C. While the qualitative advantage of this practice may be obvious, the energy gain is in fact low. The alteration of components, according to a time–temperature relationship, can also be reduced by decreasing the residence time in the facility. The physicochemical characteristics of the concentrate (non-denatured whey protein nitrogen, viscosity, insoluble mineral) depend on the length and temperature of the process and the ionic force; these characteristics largely determine the properties and qualities of the final powder.

19.3.1.1 Principle of vacuum evaporation

Single-stage vacuum evaporation consists of placing the liquid to be concentrated, which has been brought to its boiling temperature beforehand, into a vacuum chamber (evaporation body; Fig. 19.1). The vacuum, obtained by the condensation of spray in contact with a cold source, corresponds to the saturation vapour pressure at the boiling point of the product.

In this context, any heat applied to the product will result in vaporisation of some liquid. The evaporation body is

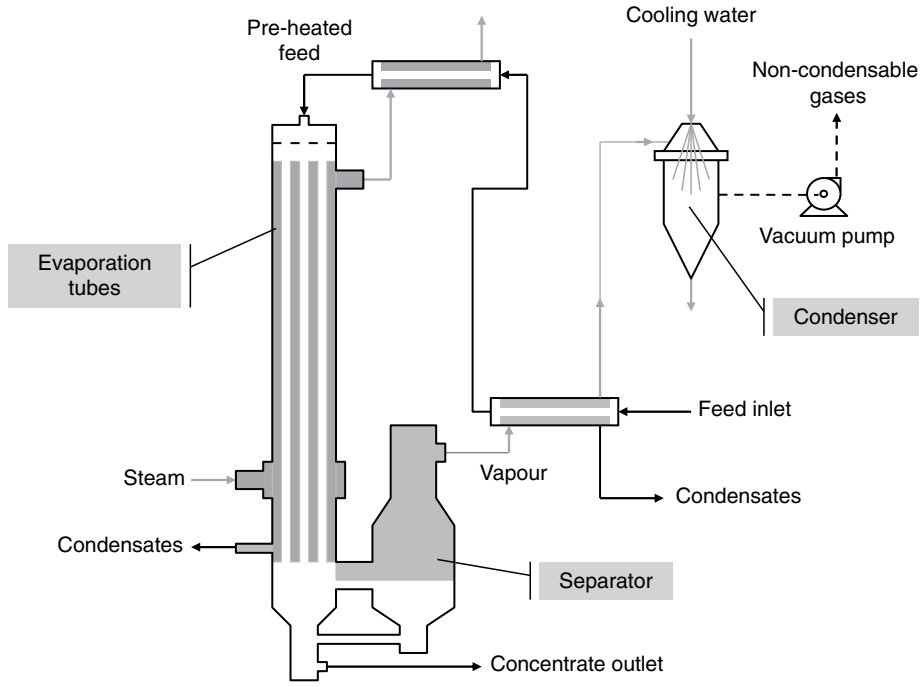


Figure 19.1. Single-stage falling-film evaporation.

thus a heat exchanger for providing the product with latent heat of vaporisation. In practice, the energy supplied to the heat exchanger (tube bundle in general) comes from vapour at a temperature 5–10°C higher than that of the product.

The liquid–vapour mixture is separated in a separation container attached to the evaporation body. In this way the secondary vapour (still called vapour spray) and the concentrated liquid is collected. The energy contained in the vapour mist is usually recovered, either to reheat the incoming product or to heat a second evaporation body. This principle of multiple-stage evaporation will be explained in greater detail later.

Each evaporation unit must meet three industry requirements: high evaporation capacity, low specific energy consumption and ability to maintain quality of the concentrate. The types of evaporator differ depending on the liquid flow or the geometry of the heating surfaces, and are more or less adapted to the different food liquids: (i) climbing film evaporators; (ii) falling-film evaporators (Fig. 19.1); and (iii) plate evaporators.

19.3.1.2 Energy

The elimination of water is often an expensive operation in the processing of liquid food. For example, the enthalpy balance of single-stage evaporation, including consideration of energy losses, highlights the following two points:

1. The evaporation of 1 kg of water from a treated product requires condensation of 1.1 kg of primary vapour. The energy cost is therefore about 2700 kJ/kg of evaporated water (Knipschildt, 1986).
2. The specific enthalpy of the secondary vapour thus obtained is slightly less than that of the primary vapour, corresponding to a temperature drop of 3–5°C.

There are several solutions to reducing the cost of concentration by vacuum evaporation. The multiple-stage evaporator consists of a set of single-stage evaporation units connected in series (Fig. 19.2), whereby the liquid food being concentrated passes from one stage or 'effect' to the other. The first stage is heated with direct steam injection while the next ones are heated with vapour mist generated in the preceding stage. The last stage is connected to a condenser, which creates a vacuum in the entire system. The energy cost of removing water in an evaporator with n stages is therefore (2700/n) kJ/kg of water removed (not taking into account sensible heat) (Knipschildt, 1986).

While a temperature difference is necessary between the heating vapour and the product to be concentrated, the evaporation temperature, and therefore the pressure, decreases from one stage to the next. The pressure gradient between the first and last stage is controlled by the vacuum pump, which is connected to the condenser that collects the

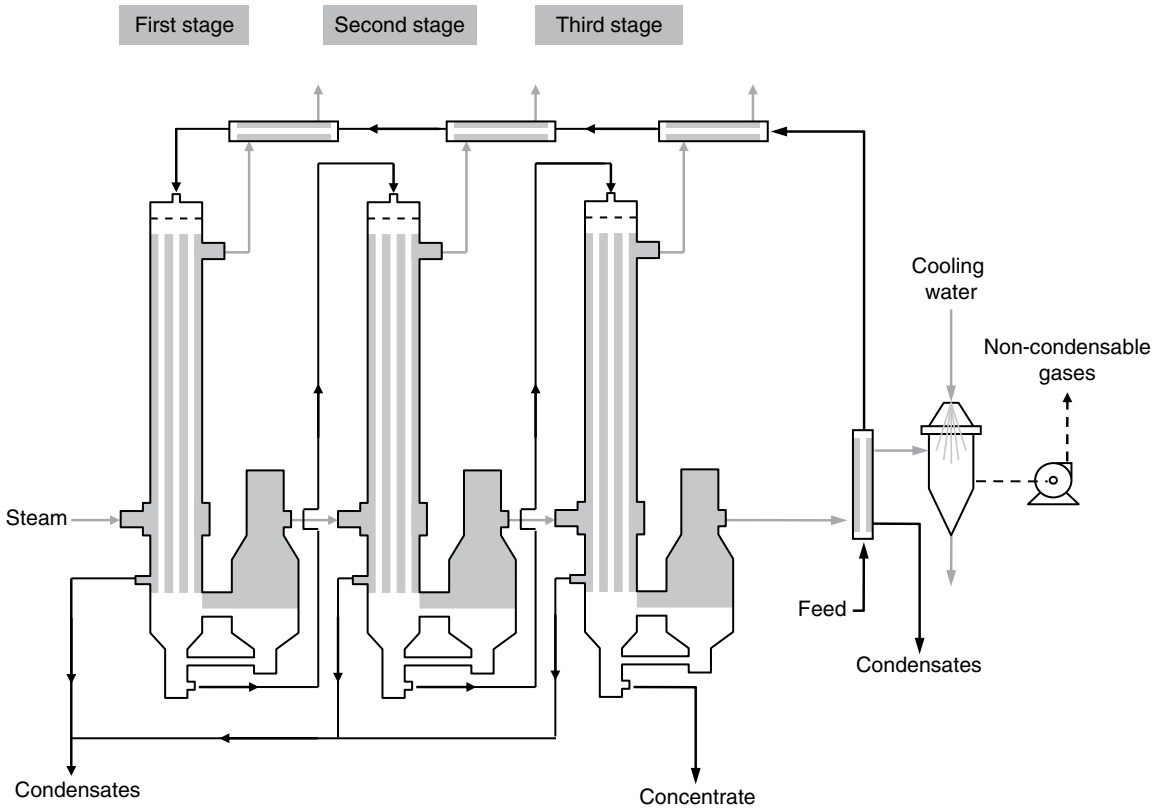


Figure 19.2. Multiple-stage evaporation.

vapour extracted from the last stage. The limits to multiple-stage evaporators are:

- the maximum temperature that the product can withstand in the first stage due to its heat sensitivity – in practice, this temperature is generally between 70 and 90°C for foodstuffs;
- the temperature in the last stage, which is limited by the temperature of the condensate and/or the increase in viscosity due to the drop in temperature and the increase in dry matter of the concentrate – in practice, this temperature is generally greater or equal to 40°C;
- the drop in the evaporation temperature from one stage to the next – this drop is generally greater or equal to 5°C (Knipschildt, 1986; Westergaard, 2004).

The compromise between energy cost reductions and investment in additional stages (depreciation and maintenance) is concentration facilities comprising three to six stages. However, modern facilities allow a greater reuse of vapour. The principle consists of compressing the vapour mist, thereby increasing its enthalpy and injecting it back into the stage where it was created. Two methods are used to do this: thermocompression (Fig. 19.3) and mechanical vapour recompression (Fig. 19.4).

Thermocompression can easily be integrated into a multiple-stage system and provides an energy gain equivalent to an additional stage at a lower investment cost. Mechanical vapour recompression, even if linked to just one stage, can significantly reduce energy costs.

To summarise, the various measures that can improve the energy costs of the evaporation operation are (i) preheating solutions, (ii) recovery of heat from condensates or concentrates and (iii) multiple-stage evaporation, coupled or not with thermocompression and mechanical vapour recompression. These factors substantially influence the energy consumption of concentration by vacuum evaporation. Even though the data in the literature are not always consistent (whether or not the following are taken into account: centrifuge pumps, vacuum pumps, condensate, vapour, boiler efficiency, etc.), energy consumption decreases by 2600–3100 kJ/kg for a single-stage and 260–330 kJ/kg for six stages with thermocompression (Kessler, 1986; Westergaard, 2004).

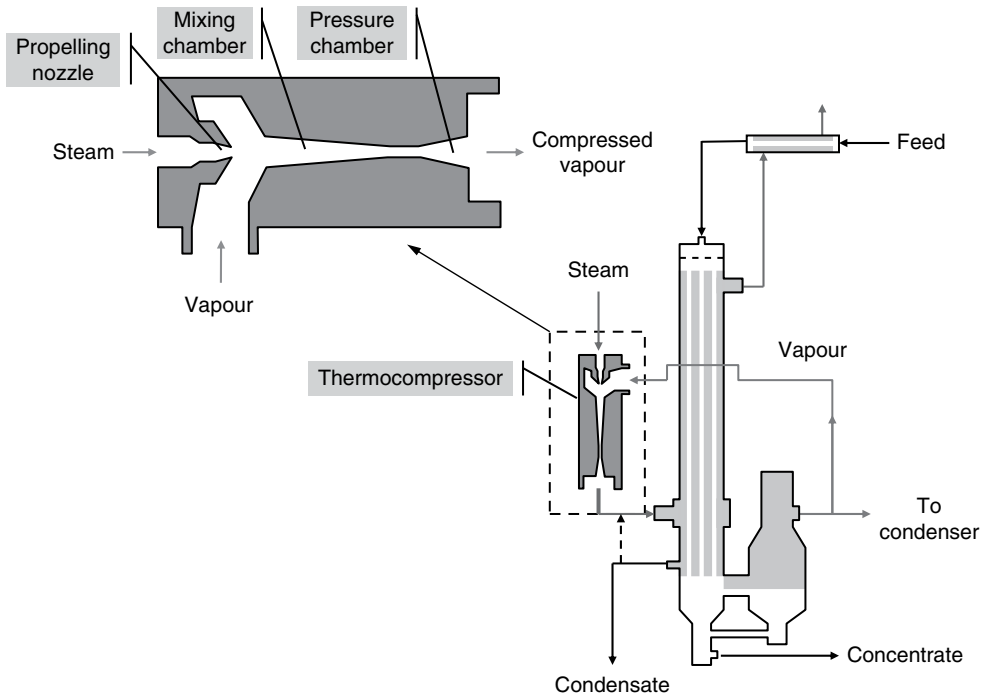


Figure 19.3. Evaporation with a steam jet compressor.

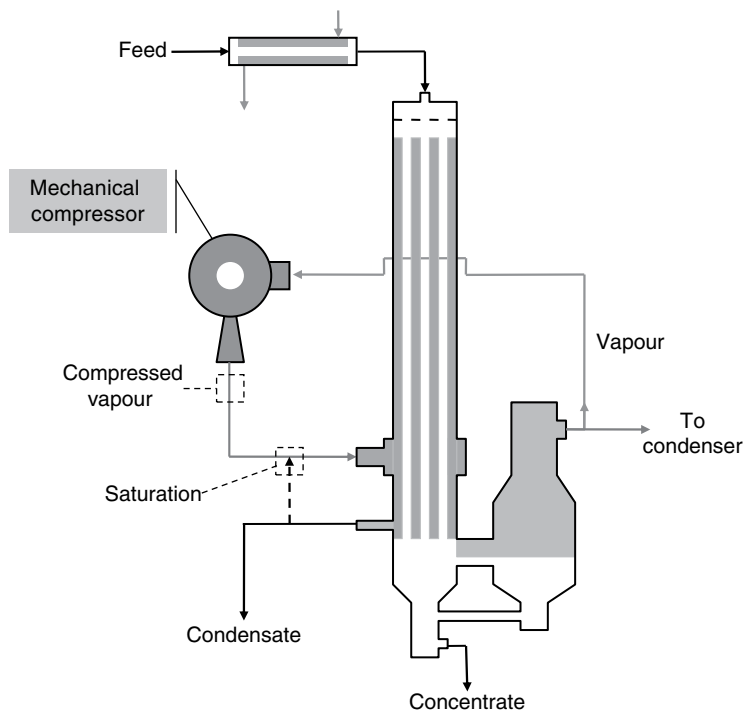


Figure 19.4. Evaporation with mechanical compressor.

19.3.1.3 Production of concentrated whole and skimmed milk

The flow chart of milk concentrates and milk powder production includes milk reception, clarification, cooling, standardisation, heat treatment, concentration mainly by evaporation, and homogenisation, followed by either drying for powder or sterilisation for concentrated milk. Raw full-fat milk to be used for powder production must be of high chemical, sensory and bacteriological quality and must meet the standards approved by governments in many countries. At reception, milk is clarified, usually by centrifugal separators, cooled to 4°C in plate heat exchangers and stored at the same temperature. After standardisation or skimming, which is used to adjust the ratio of milk fat to total solids as required in the final product, milk is heated using tubular or plate heat exchangers. This heating serves to destroy pathogenic microorganisms, inactivate enzymes (especially lipase) and inhibit oxidative changes (i.e. by activating the SH groups in β-lactoglobulin, which results in an antioxidative effect). Heating (i.e. temperature–time relationship) is determined by the specificity of the final product. For evaporated milk, long heat treatment (10–25 min) at a temperature below 100°C is often applied to enhance the heat stability of the milk, but currently ultra-high-temperature (UHT) processing is preferred. UHT heating is also commonly applied for sweetened condensed milk (Walstra *et al*., 2006). However, for milk powder, heat treatment is commonly performed at 85–95°C for 15–30 s because the shelf-life of the product is extended by the decrease in water activity by the high dehydration level.

After the heat treatment stage, the milk is usually concentrated by evaporation, using multiple-effect vacuum evaporators with mechanical and thermal steam recompression, where energy consumption is about 10–30 times lower than in spray drying (Schuck, 2011a). After concentrating the milk solids, homogenisation is applied to prevent creaming and coalescence of the fat content in the concentrated milk. Homogenisation also reduces the 'free-fat' content in whole-fat milk powder, resulting in increased powder solubility and a decrease in oxidation phenomena (Schuck, 2011a).

The procedure for the manufacture of concentrated skimmed milk and powders differs in several respects from similar products made from whole milk. First, standardisation of the fat content leads to a very low fat level in the skimmed milk (0.05–0.10 g/100 g). The homogenisation stage is omitted, and the skimmed milk is concentrated to a higher solids content; however, concentration of skimmed milk using reverse osmosis can be achieved to a maximum total solids content of 30 g/100 g. Moreover, the regimen of skimmed milk heat treatment may be more intense than that of whole milk, and may be adapted according to the intended applications of the powder being produced; these aspects are known as 'heat specification'. Low-heat milk powder, which is used in the production of cheese, baby foods, etc., is usually obtained by simple pasteurisation (i.e. 72°C for 15 s).

High-heat milk powder requires additional heat treatment, such as 85–88°C for 15–30 min (Schuck, 2011a). This intense heat treatment is generally applied in the production of SMPs used in the bakery industry, chocolate industry, and other foods where a high degree of protein denaturation, i.e. low whey protein nitrogen index(WPNI), is desired. Medium- and high-heat milk powders are used in cheese manufacture, but they result in most of the problems encountered in cheesemaking. The cumulative effects of heat treatments applied for purposes of microbiological control and thermal efficiency during concentration and drying considerably affect the cheesemaking abilities of the resulting powders. In that context, membrane technology, particularly microfiltration (MF) offers alternatives in order to improve the cheesemaking potential of skimmed milk concentrates and powders.

19.3.1.4 Production of dulce de leche

Dulce de leche (milk jam, milk caramel) is a form of sweetened condensed milk that is very popular in South American countries such as Argentina, Uruguay, Brazil and Mexico, and may be produced from cow or goat milk. There are two main types of *dulce de leche* product: the first, for household use, is consumed as a spread or a dessert, and the second, for confectionery use, has a higher viscosity. The industrial manufacturing stages of these products are similar to those of sweetened condensed milk, where the milk solids are concentrated by heating and evaporation. In the particular case of *dulce de leche*, sucrose is added to the milk prior to concentration until 70% total solids is reached.

The most significant technological problem in *dulce de leche* production is the sandy texture of the product, which has a negative impact by reducing product acceptability. This phenomenon is mainly attributed to the high concentration of lactose in the product leading to crystallisation; the lactose crystals can be up to 1500 μm in size. Considerable effort has been devoted to resolving this problem, by breaking down lactose using bacteria or enzymes, by seeding with lactose microcrystals or by using ultrafiltration in order to reduce the processing time and lower the final lactose content of the milk (Oliveira *et al*., 2009).

19.3.2 Whey and lactose crystallisation

Whey is the liquid dairy product obtained during the manufacture of cheese, casein or similar products through

separation of the curd following coagulation of the milk and/or the derivative products of milk. Whey was for many years regarded as a by-product, the use of which was limited to animal feed and fertilisers. Today, whey is a valuable source of ingredients for the food and pharmaceutical industry. The quantity of whey released corresponds to nine times the weight of the final cheese manufactured, and whey contains not less than 50% of the dry matter of the milk transformed into cheese.

Although conventional milk powder dehydration is quite well known on an industrial scale, development of new products produced from qualitatively different whey sources has given rise to a number of problems in drying and conservation, mainly due to amorphous lactose. A prior step of lactose crystallisation in concentrated whey is thus necessary. Additionally, between the evaporation and drying steps, whey powder manufacture includes a lactose crystallisation step that is often performed in a stirred tank over a few hours. Lactose crystallisation in whey is a key stage in the manufacture of whey powders. Controlling this stage at an industrial level should increase the prospects of improving the process and also the physicochemical qualities of powders. It is essential to control this step in order to obtain specified and reproducible powders, in terms of size and crystallisation level, whatever the initial chemical composition. The lactose crystallisation process is a key stage in the processing of whey and lactose powders. Controlling this appears to be fundamental for the drying stage and storage since the transition of lactose to a crystallised form decreases the proportions of hygroscopic compounds (amorphous lactose) (Roos & Karel, 1992; Bhargava & Jelen, 1996; Bhandari *et al*.,1997).

Lactose crystallisation occurs in highly supersaturated solutions, indicating that the phenomena of nucleation and crystal growth can take place simultaneously. In addition, a preliminary stage of mutarotation between anomeric forms is added in the case of lactose, each with their own kinetics.

At an industrial level, lactose crystallisation is also performed in a medium of complex chemical composition. In particular, certain macromolecules are also present and the influence of solids such as the whey proteins on the kinetics of lactose crystallisation has received very little attention (Mimouni *et al*., 2005, 2007, 2009).The literature indicates that the kinetics can be modified by the presence of other components at each change of state. In order to understand their influence fully, it is necessary to understand their specific effects on lactose solubility as well as the laws of the kinetics controlling the stages of mutarotation, nucleation and growth (Gernigon*et al*., 2009, 2010; Schuck, 2011b).

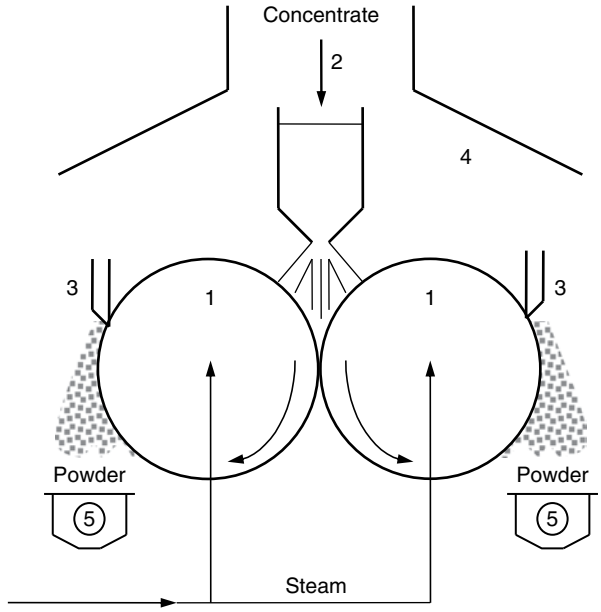


Figure 19.5. Roller dryer: 1, drum; 2, feed pipe; 3, knife; 4, vapour hood; 5, conveyor.

19.3.3 Drying

Dehydration of food products can ensure good stability by lowering the a_w and reducing transport and storage costs (Bimbenet & Loncin, 1995). It can be done as follows:

- By evaporation at boiling temperature at atmospheric pressure or under partial vacuum, drying by direct contact on heated rollers for example (Fig. 19.5) (this method is not covered in this chapter).
- By sublimation of ice at partial pressures of water below the triple point (610.8 Pa), corresponding to the direct transition from solid to gaseous state (this method, corresponding to freeze drying of food products such as coffee and mushrooms, is not covered in this chapter).
- By the combined action of a heat transfer of hot air to the product and a water transfer from the product to the hot dry air (spray drying for example).

19.3.3.1 Spray drying

19.3.3.1.1 Principles

Spray drying (or atomisation) is a particle drying technique. It involves spraying the product, which is in liquid form or in suspension, into a hot gas stream. This is without a doubt the most used drying method for all food sectors combined (e.g. charcuterie, fish products, fodder, cereals and vegetable products, fruit, milk, eggs, blood). Several techniques are involved in this method.

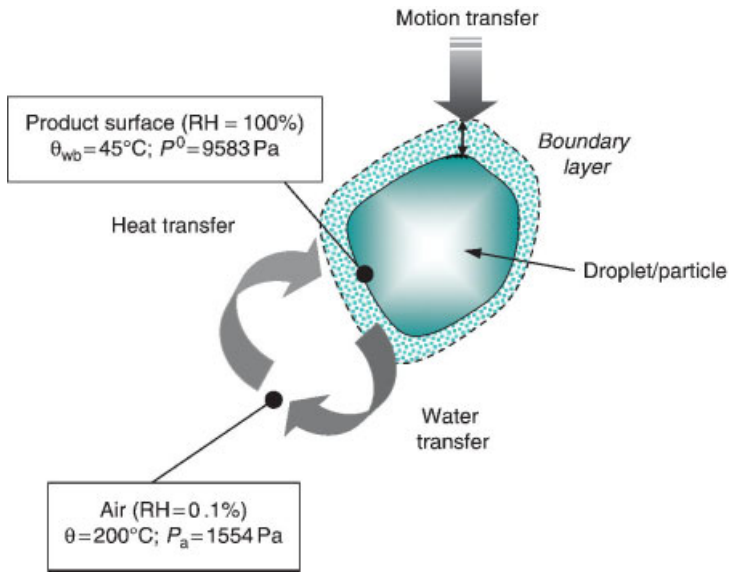


Figure 19.6. Principles of spray drying. P^0, saturation vapour pressure (see Equation 19.3); P_a, vapour pressure of air; θ_{wb}, wet bulb temperature; RH, relative humidity.

Spray drying involves entrainment. When a wet product is placed in a sufficiently hot and dry stream of air (or another gas), a temperature and partial water pressure gradient spontaneously occurs between the product and the air causing:

- heat transfer from the air to the product due to the temperature difference;
- reverse water transfer due to the difference in partial water pressure between the air and the surface of the product (Fig. 19.6).

The air therefore serves as both a heat transfer fluid and a carrier gas for the elimination of water vapour. The air is hot and dry when it enters the drying tower and cold and wet when it leaves. The surface temperature of the product is equivalent to the wet air temperature, that is around 45°C for dry air at 200°C (Bimbenet & Loncin, 1995).

Drying is a method of evaporation of surface water resulting from the capillary rise of water inside the droplet towards the surface. As long as the average humidity is sufficient to keep the surface sufficiently wet, the speed of evaporation will be constant; otherwise it will decrease and the surface temperature will rise. The drying speed is proportional to three factors:

1. the evaporation surface, which increases as the diameter of the droplets decreases – dehydration speeds up as the interface, created by spraying the liquid and hot air, increases in size, thereby minimising heat damage to the product (Pisecky, 1997);
2. the difference in partial water vapour pressures between the surface of the particle and the air, which depends on the AH and the inlet air temperature;
3. the rate of water migration from the interior to the surface of the particle, which can be reduced by surface crusting due to heat denaturation of some components.

19.3.3.1.2 Equipment design

The main components of a spray-drying installation, defined according to Pisecky (1997), Masters (2002) and Westergaard (2002), are shown in Fig.19.7. They include the drying air system, the concentrate spraying system and the drying chamber.

Drying air

The suction of atmospheric air is carried out by filters (Fig. 19.7, position 17), the type of which depends on local conditions and the nature of the product to be treated. The air can be heated (Fig. 19.7, position 5) in two different ways: by direct heating (gas or electricity) and/or by indirect heating (steam, gas, oil).

1. *Direct heating* using gas involves contact between the air to be heated and the combustion products. This process, which is the least expensive, has a number of advantages (high efficiency, low inertia, fine tuning,

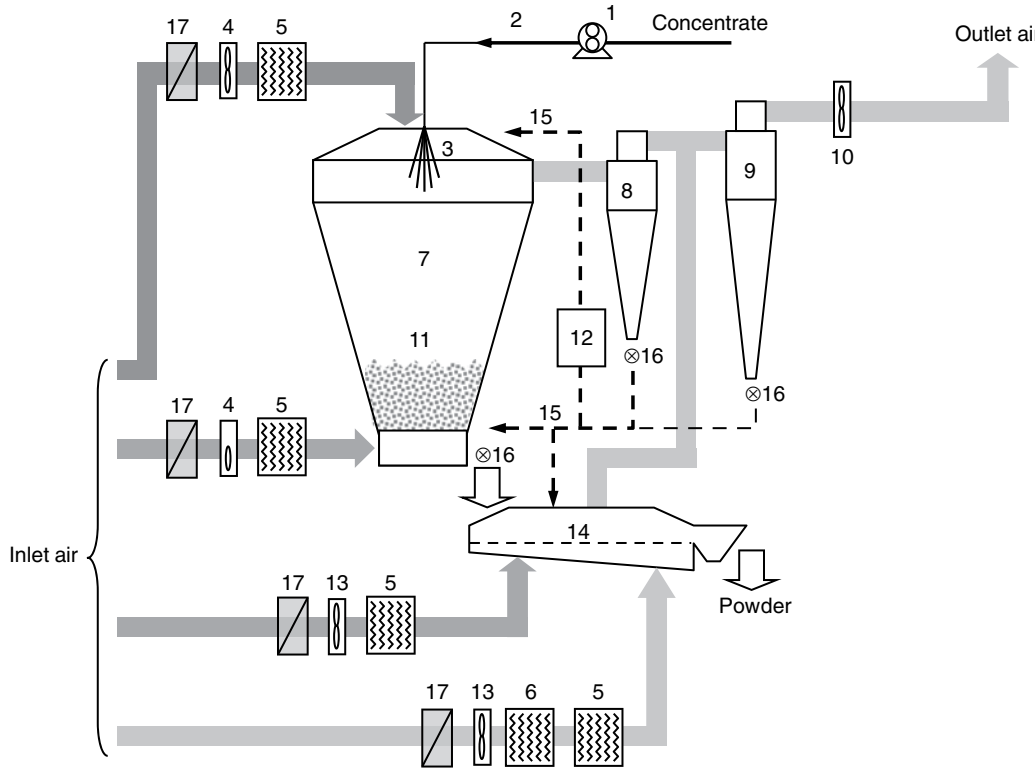


Figure 19.7. Multiple-stage spray-drying installation: 1, feed pump; 2, feed flow; 3, sprayer/air disperser; 4, inlet air fan; 5, air heater; 6, air cooler; 7, drying chamber; 8, primary cyclone; 9, secondary cyclone; 10, outlet air fan; 11, integrated fluid bed; 12, pressure conveyer system; 13, vibro-fluidiser air fan; 14, vibro-fluidiser; 15, reincorporation of fines; 16, rotary valve; 17, air filtration.

temperatures of up to 400°C, low investment, small footprint). The major drawback is the production of water from the combustion of methane, between 40 and 44 mg/kg air per °C, which reduces the evaporation capacity of the facility by 20–25% (Pisecky, 1997; Masters, 2002; Westergaard, 2004). In addition, the combustion of gas results in the production of nitrogen oxides, which contaminates the powders; therefore direct heating of the air with gas is hardly used in the manufacture of powders for human consumption.

2. *Direct electric heating* of air has the same advantages as gas heating without the combustion of water, but results in higher energy costs. For this reason, industrial drying facilities generally use a mixed heating system with heater batteries (indirect) and electric booster batteries (fine tuning and low inertia).
3. *Indirect heating* using batteries containing a heat transfer fluid (steam, gas, oil). These batteries are usually situated in the sheath between the ventilator and the air disperser. The advantages of this heating system are the absence of contact between the heat transfer fluid and the product, and the absence of combustion water. The drawbacks are mainly energy-related. In reality, it is less efficient than direct heating (between 70 and 90% taking into account the efficiency of the boiler and the heater batteries), heating temperatures are lower (<250°C) and investment is greater. However, this type of air heating is often used in the agri-food industry (Pisecky, 1997; Masters, 2002; Westergaard, 2004). The distribution of air is achieved by means of an inlet distributor, located mostly at the top of the drying chamber. The mixture between drying air and concentrate droplets is optimal when the spray system is positioned at the centre of the air disperser.

Spray system

The primary functions of atomisation are (i) to produce a high surface to mass ratio, resulting in a high evaporation rate; and (ii) to produce particles of desired shape, size and density.There are three types of sprayers (Fig. 19.7, position 3):

Table 19.1. Advantages and disadvantages of various atomising devices.

	Advantages	Disadvantages
Wheel	Flexibility, high flow rate, high viscosity and high total solids concentrate	Investment (wheel) and maintenance costs of atomising device, increase in occluded air and decrease in powder density
Pressure nozzle	Decrease in occluded air and increase in powder density, improvement of the flowability, enables agglomeration, low investment cost (orifice and grooved core inserts)	Maintenance costs of the high-pressure pump, early wear of core and orifice with dispersed elements (crystals, etc.), problems with concentrate at high total solids and viscosity
Two-fluid nozzle	Pressure-sensitive products	Increase in occluded air and decrease in powder density

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1. *Rotary atomiser*: the liquid, which is carried to the centre of the wheel, is ejected by centrifugal force. The rotation speed (wheel or disc) is 10 000–25 000 rpm. The quantity of liquid injected can reach 75 000 kg/hour (Masters, 2002).
2. *Liquid pressure nozzle*: the liquid passes through an orifice insert located above the inlet distributor. The dispersion energy is released by expansion of the liquid, pumped under pressure. Depending on the facility, the pressure can vary from 5 to 30 MPa. The quantity of product passing through a high-pressure nozzle varies between 1000 and 1500 kg/hour (Masters, 2002).
3. *Two-fluid nozzle*: the atomisation energy is released by expansion of compressed air. This nozzle can spray products that cannot withstand high pressure.

Table 19.1 summarises the advantages and disadvantages of the three spray systems.

Drying chamber and powder recovery system

The drying chamber (Fig. 19.7, position 7) is cylindrical or cylindro-conical depending on the facility and type of sprayer. The principle of a 'single-stage' atomisation unit is based on the fact that drying occurs entirely in the chamber. The residence time in the drying tower is too short (20–60 s on average) to achieve an equilibrium between the relative humidity (RH) of outlet air and the water activity of the powder, which reduces the thermal efficiency of the unit (Table 19.2). As a result, single-stage spray driers are now considered outdated.

'Two-stage' drying involves limiting spray drying and continuing with a process that lasts longer (several minutes), and is therefore closer to the thermodynamic equilibrium. The product leaving the spray-drying unit should have a maximum moisture content compatible with continuous discharge, and acceptable operating conditions should be maintained. The air leaving the tower has a higher moisture content and a lower temperature, which improves energy efficiency and allows higher inlet air temperatures (Table 19.2). A second final drying stage is necessary to optimise the final moisture content by using an integrated (static) fluid bed or an external (vibrating) fluid bed (Fig. 19.7, positions 11 and 14), whereby airflow and temperatures are lower than in the chamber and therefore better designed for the qualitative preservation of powders. The integrated fluid bed can be either circular (for example multiple-stage dryer (MSD™)), or annular (compact dryer).

The principle of two-stage drying clearly shows how to reduce drying costs and improve unit performance: transfer most of the drying from the 'atomisation' to the 'fluidisation' stage until the wet product starts to stick to the walls of the chamber. Sticking occurs when the product temperature is slightly higher than its glass transition temperature (T_g), which gives it a thermoplastic behaviour. In order to overcome this difficulty, three-stage drying systems, with an internal fluid bed as a second stage and an external vibrating fluid bed as a third stage, first appeared at the beginning of the 1980s and were called 'compact dryer instantisation'(CDI) or MSD™. Three-stage systems combine all the advantages of extended two-stage drying, using spray drying as the primary stage, fluid bed drying of a static fluid as the second stage and drying on an external vibrating fluid bed as the third stage. Two-stage and three-stage drying may produce both non-agglomerated and agglomerated powders.

There are other types of dryers such as compact towers (tower W), flat-bottom towers, 'Tall Form', Filtermat®, Paraflash® or Tixotherm®. The kind of tower dryer depends on the specific properties of the product to be dried (high fat content, starches, maltodextrins, egg products, hygroscopic products, etc.).

Whatever type of drying tower is used, most of the product is collected at the bottom of the drying chamber; however fine particles are carried away by the air. In general,

Table 19.2. Operating parameters and energy efficiency of various spray-drying processes.

		Spray dryer (SD)			
Drying system	**Unit**	**SD one-stage**	**SD with VF**	**SD with SFB (Compact)**	**SD with SFB (MSD™)**
Spray dryer					
Inlet air temperature	°C	200	230	230	260
Drying air	kg/h	31 500	31 500	31 500	31 500
Skimmed milk with 8.5% solids	kg/h	12 950	19 800	24 000	31 300
Concentrate with 48% solids	kg/h	2290	3510	4250	5540
Evaporation in chamber	kg/h	1150	1720	2010	2620
Powder from chamber					
3.5% moisture	kg/h	1140	—	—	—
6.0% moisture	kg/h	—	1790	—	—
9.0% moisture	kg/h	—	—	2240	2920
Fuel oil consumption	kg/h	175	205	205	230
Power consumption	kW	120	130	140	150
Energy consumption:					
Spray drying total	MJ	7612	8876	8918	9965
Energy/kg powder in chamber	kJ	6677	4959	3981	3413
Fluid bed (FB)		—	*VF*	*SFB*	*SFB*
Inlet air temperature	°C	—	100	115	120
Drying air	kg/h	—	4290	6750	11 500
Evaporation in VF/SFB	kg/h	—	45	125	165
Powder from FB, 3.5% moisture	kg/h	—	1745	2115	2755
Steam consumption	kg/h	—	167	290	400
Power consumption	kW	—	20	25	35
Energy cons., total in fluid bed	MJ	—	481	816	1110
Drying total					
Energy consumption total	MJ	7612	9357	9734	11 075
Energy per kg powder total	kJ	6677	5362	4602	4020
Energy ratio	%	100	80	69	60
Dryer efficiency	–	0.54	0.66	0.69	0.79

MSD, multi-spray-dryer; SFB, static fluid bed; VF, vibro-fluidiser.

Sources: reproduced from Schuck *et al*. (2012), with permission of John Wiley & Sons, Ltd., including further data from Bimbenet *et al*. (2002), Carić (2002), Westergaard (2004) and Jeantet *et al*. (2008a, b).

these particles are separated from the outlet air by cyclones (Fig. 19.7, positions 8 and 9) or bag filters. Final cleaning of the exhaust air is carried out using a wet scrubber and dry filter in order to limit emissions into the atmosphere. European standards indicate that these emissions should be less than 40 mg/m^3 of air at 20°C (Schuck *et al*., 2012). Depending on the drying units and their configuration, the fine particles can be reincorporated either via an internal or external fluid bed or via a spray system. Of course, these different possibilities considerably influence the physical properties of powders.

The optimisation of the process, free from any initial constraints, led to a remarkable improvement in performance and better product quality. The advantages of this technique are as follows:

- Improvement in energy efficiency (see Table 19.2).
- Reduced size: in a given volume, the capacity is two to three times higher than that of a conventional unit.
- Significant reduction of air emissions: decreasing the flow of drying air and increasing the moisture content of the product reduces loss by entrainment.

- Improvement in product quality with regard to agglomeration, solubility, dispersibility, wettability, particle size, density, etc.

19.3.3.1.3 Energy

The energy performance of spray-drying installations can be evaluated based on different criteria. The mass energy consumption (MEC), in kJ/kg of evaporated water, is the amount of heat supplied per unit mass of water dried. It is also possible to determine the ratio *R* between the actual energy used and the energy cost (Bimbenet & Loncin, 1995; Bimbenet *et al*., 2002):

$$R = \frac{\theta_i - \theta_o}{\theta_i - \theta} \qquad (19.1)$$

where θ_i represents inlet air temperature after heating, θ_o outlet air temperature and θ ambient air temperature.

The calculation of MEC is based on the enthalpy of humid air and is more precise than the *R* ratio, based solely on the temperature differences of used air used without taking into account the RH of such air. However, for a rapid estimation of the energy costs incurred by spray drying, the *R* ratio can be used.

Table 19.2 shows the energy performance of different spray-drying configurations in the production of skimmed milk powder using a 48% dry matter concentrate: the energy consumption per unit mass is 6677, 5362, 4602 and 4020 kJ/kg for a single-stage dryer, a two-stage dryer with vibro-fluidiser, a compact two-stage dryer with static bed, and an MSD™ dryer with static bed, respectively. This improved performance can be explained by that fact that by increasing the number of drying stages, the residence time simultaneously increases thereby allowing an increase in the inlet air temperature, the concentrate flow rate and ultimately energy efficiency for higher or equal quality powders (Bimbenet *et al*., 2002; Carić, 2002; Westergaard, 2004; Jeantet *et al*., 2008a).

19.3.3.1.4 Control and improvement of powder properties

Owing to the variety and complexity of the mixes to be dried, a more rigorous method based on physicochemical and thermodynamic properties has now become necessary. Improving understanding of the biochemical properties of milk products before drying, water transfer during spray drying, the properties of powders and influencing factors has now become indispensable in the production of dairy powder. Lack of technical and economic information and of scientific methods prevents the manufacturer from optimising the process in terms of energy costs and powder quality. Two approaches are necessary in dairy research on spray drying of dairy products, one involving the products (availability of water related to the biochemical composition) and the second involving the process (understanding and improvement of the drying parameters).

Availability of water

The aim of this section is to propose a new method (drying by desorption, using a thermohygrometer sensor) in order to determine major drying parameters according to food components in relation to their interactions with water (bound and free water) and linked to water transfer kinetics. The studies by Schuck *et al*. (1998, 2009) have shown that drying by desorption is an excellent tool to determine and optimise the major spray-drying parameters in relation to biochemical composition according to water availability and desorption behaviour (calculation of extra energy ΔE). The experimental device proposed by the authors differs from spray-drying equipment in terms of duration of drying, drying temperature, surface–volume ratio, etc., because the concentrate is dried in a cup and not in a droplet. However, computational tools have been developed to improve the method by taking these factors into account. Validation tests (>80 products) have indicated that this method could be applied to a wide range of food products and spray-dryer types. For reasons of calculation speed and reliability, this method has been computerised and it can already be used in the determination of parameters of spray drying for food products. The name of the new software is Spray Drying Parameter Simulation and Determination Software ($SD^2P^{®}$) registered under the following identification number: IDDN.FR.001.480002.003.R.P.2005.000.30100.

Analysis of the desorption curve (measured RH vs. time), combined with knowledge of the temperature, total solids, density and specific heat capacity of the concentrate, airflow rates, theoretical water content in relation to water activity and RH of the outlet air, the current weather conditions, cost per kilowatt hour and the percentage of drying in the integrated fluid allows determination of enthalpy, temperature, RH (including ΔE) for inlet air, concentrate and powder flow rate, specific energy consumption, energy and mass balance, yield of the dryer and cost (in euros or US dollars) to remove 1 kg of water or to produce 1 kg of powder. All these results are summarised in Fig.19.8. This figure is a representation of the software delivery of (i) the air characteristics at the dryer/integrated fluid bed inlet and outlet (upper part) and (ii) the flow, energy and cost calculations (lower part) (Schuck *et al*., 2009).

Process improvement

The aim of this section is to show the use of a thermohygrometric sensor, with some examples of such measurements [temperature, absolute humidity (AH) and RH, dry air flow rate, water activity] through calculation of

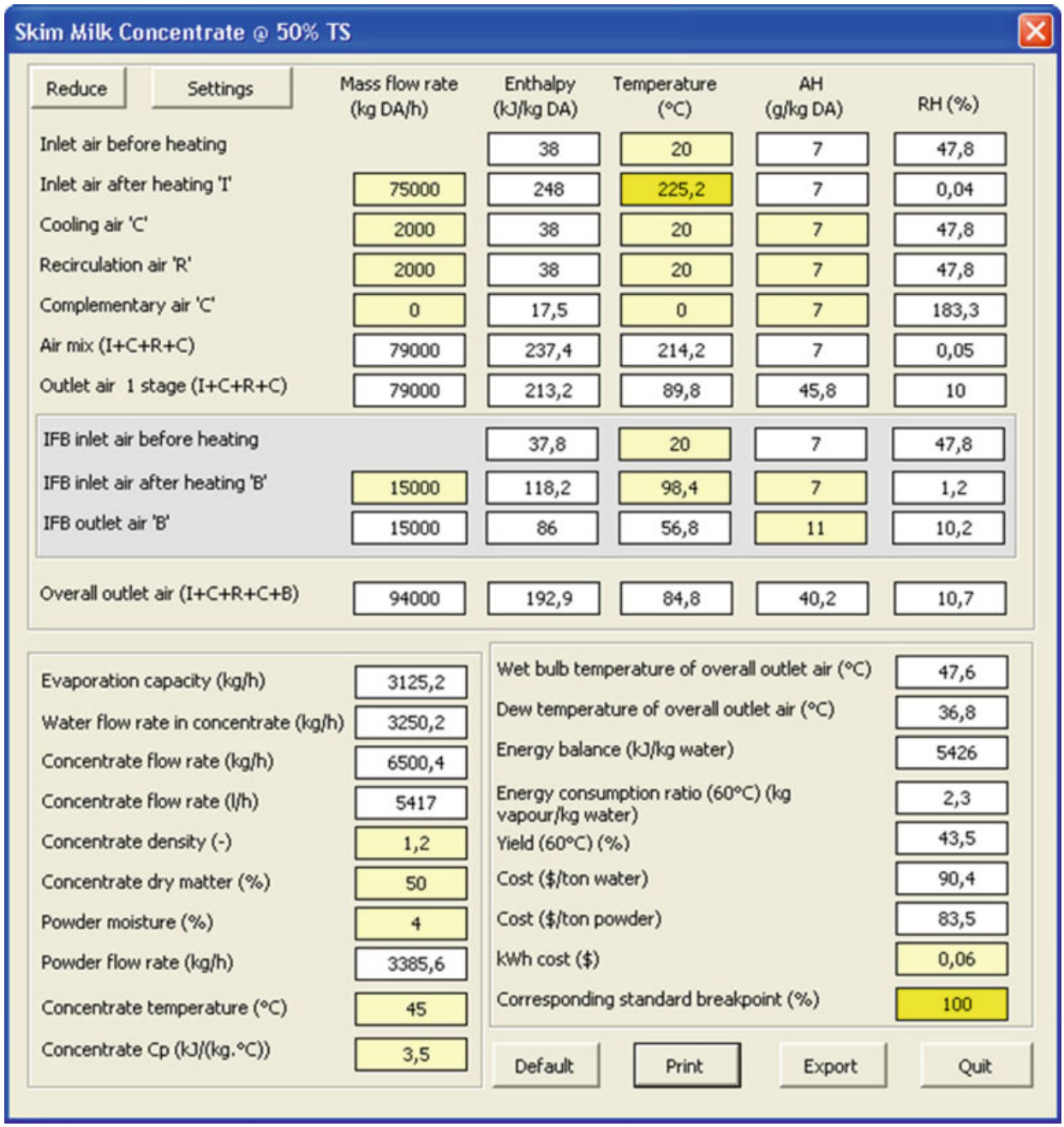


Figure 19.8. Parameters of spray drying calculated by the SD²P® software.

mass and AH, to prevent sticking in the drying chamber and to optimise powder moisture and water activity in relation to the RH of the outlet air.

It was demonstrated by Schuck *et al*. (2005a) that a thermohygrometer can be used to avoid sticking and to optimise water content and water activity in dairy powders. It can be seen from these results that the calculated AH is systematically higher than the measured AH, because the calculated AH corresponds to the maximum theoretical value that can be reached. Calculation of AH by means of

the mass balance is based on the hypothesis that the air circulating in the spray drier removes all the water from the concentrate. Thus, if the difference between the calculated and measured AH of the outlet air is below 2 g of water/kg dry air (depending on the measurement accuracy of the spray drier), there is no problem of sticking in the spray dryer chambers, whatever the dairy concentrate used. However, sticking was observed in this study for differential levels of AH above 2 g water/kg dry air, corresponding to lower water removal and consequently favourable to sticking conditions. The operator can follow the AH and anticipate a variation in drying parameters according to the differences between the calculated and the measured AH.

The operator can also follow the RH in the outlet air. To achieve a dairy powder with the same water activity and moisture content, the operator must always maintain the same RH in the outlet air by using the above equations according to each dairy product, whatever the spray drying conditions (inlet air temperature, RH and AH).

The changes in RH and AH (resulting from variations in AH of inlet air, total solid content of concentrate, crystallisation rate, outlet air temperature, etc.) can be rapidly observed in the outlet air using a thermohygrometer before such changes significantly affect powder moisture, water activity and powder sticking behaviour.

19.4 PROPERTIES OF DEHYDRATED PRODUCTS

Concerning the main properties of dehydrated products, a distinction is made between, on the one hand, general properties, a category encompassing biochemical, physical (water availability), microbiological and sensory properties, and on the other hand properties affected by the process, a category encompassing functional properties and properties of use. The latter are particularly important when powders are rehydrated before use in the manufacture of various food products. All these properties constitute the criteria for assessing the quality of powders and are evaluated according to internationally standardised methods (Pisecky, 1986; American Dairy Products Institute, 1990; Masters, 2002). The properties that determine the final quality of powders, and are consequently at the source of most of the defects encountered, are powder structure, solubility, water content, the presence of scorched particles, flowability, floodability, oxidative changes, flavour, colour and microbial contamination (Carić, 2002).

The quality of powders depends on numerous factors including the quality of the product before drying (composition and physicochemical characteristics, viscosity, heat-sensitive components and water availability), drying conditions (type of dryer, spraying using a nozzle or wheel, pressure, agglomeration, thermodynamic parameters and speed of the drying air) and storage conditions. Many scientific studies have been published on the influence of technological parameters on the properties of powders (Baldwin *et al*., 1980; Pisecky, 1980, 1981, 1986; Masters, 2002; Jeantet *et al*., 2008b). In particular, the qualitative differences are significant when comparing powders obtained by roller drying or spray drying. Roller drying does not always meet the quality criteria generally required (solubility >99%, dispersibility >95%, low protein denaturation and low free-fat content, etc.) due to heat stress (heating temperature between 120 and 130°C) and the length of residence time (2–20 s). Sometimes, the physicochemical changes induced by thermal shock are desired: this is the case for milk powders destined for chocolate and biscuit manufacture, where the properties of use have been improved by the increased free fat content or by Maillard reaction products. Milk powders obtained using heated rollers actually have lower values of solubility (94.6%), dispersibility (88%) and non-denatured WPNI (7 g of nitrogen per kilogram of powder) than those obtained using spray drying (Schuck *et al*., 1994).

19.4.1 Biochemical and physicochemical properties

The biochemical and physicochemical properties of powders depend mainly on the technological parameters implemented during processing. The overriding factors are water content and water availability, which is characterised by the water activity (a_w) and glass transition (T_g) of the powder. They determine the kinetics of the different reactions during the process (sticking) and/or within the powder (carbohydrate crystallisation, Maillard reaction, caking, etc.).

19.4.1.1 Water content

The water content, or humidity, of a powder is defined by the weight loss of the product following the drying procedure (103±2°C) and is expressed as a percentage of weight. For example, the maximum residual water content should be 4% for skimmed milk powder and 2.5% for WMP with 40% fat (Jeantet *et al*., 2008b). This level may of course differ depending on the specifications of the client.

The water content has a significant influence on the storability of the powder, which correlates to the water activity a_w. The thermodynamic characteristics of the drying air (depending on the technological parameters of atomisation and the physicochemical properties of the concentrate before atomisation) strongly influence the water content of a powder (Fig. 19.9). With a constant airflow, a variation in temperature of 10°C or 3.6 g of water per kilogram of dry air for inlet air or 1°C for outlet air leads to a variation of 0.2% in the humidity of the powder (Pisecky, 1997). Another key parameter for controlling the humidity

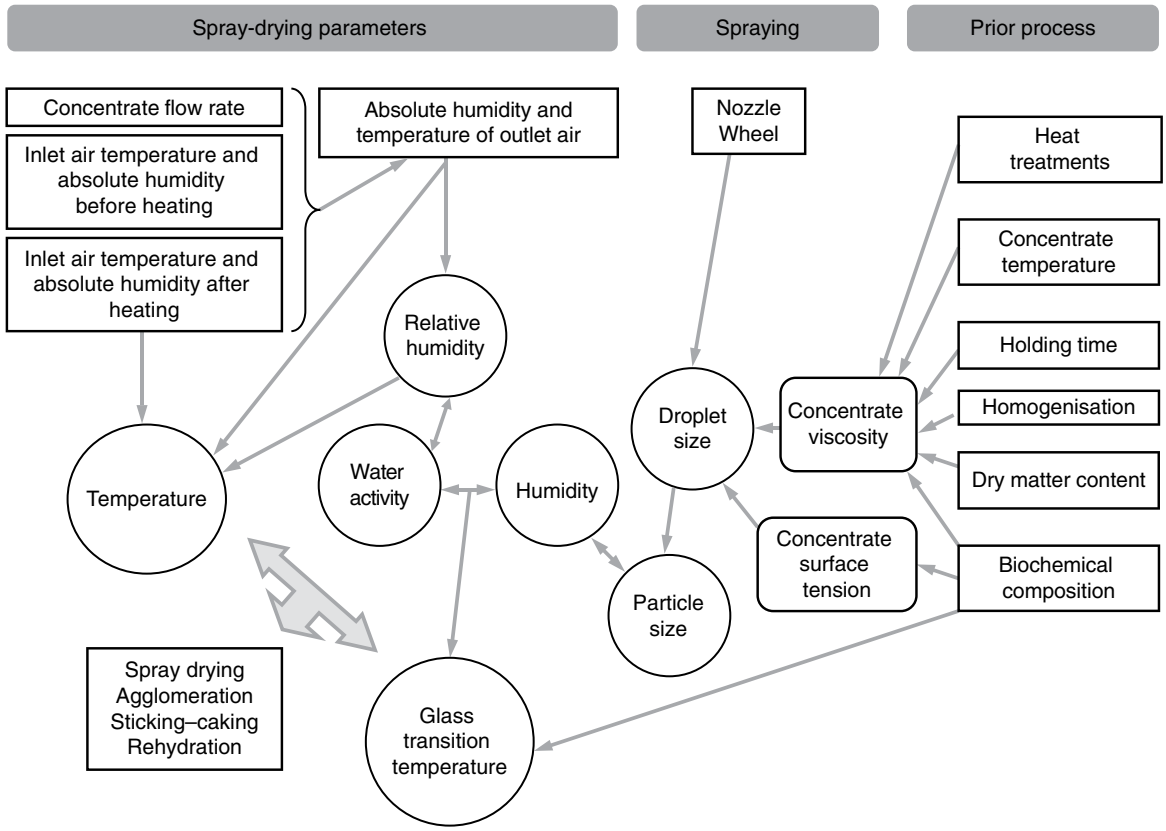


Figure 19.9. Influence of processing parameters and physicochemical characteristics on powder water content. Reproduced from Schuck *et al.* (2012), with permission of John Wiley & Sons, Ltd., including further data from Pisecky (1997).

of powders is the droplet size. An increase in the diameter of the droplets results in a decrease in the surface–volume ratio, and consequently affects the drying kinetics; for a given residence time in the atomisation chamber, a larger droplet would ultimately result in a more humid powder particle. The droplet size depends on the atomisation conditions (spray type: nozzle or wheel) and/or physicochemical characteristics of the concentrate (Fig. 19.9). In the case of spraying, enlarging the droplet size using a nozzle system is done either by increasing the size of the nozzle orifice or decreasing the spray pressure; with a wheel system, it is sufficient to decrease the speed of the wheel or increase the wheel diameter. Of the physicochemical characteristics of the concentrate, viscosity is the most influential. Thus, all the parameters that influence viscosity (temperature of the concentrate, pasteurisation conditions, concentrate dry matter, homogenisation conditions, etc.) have an indirect impact on the droplet size and therefore on the final humidity of the powder.

19.4.1.2 Water availability

19.4.1.2.1 Water activity

The a_w of a product characterises the water availability as a solvent or reagent. It is defined as the ratio between the water vapour pressure of the product (P_p) and the vapour pressure of pure water (P_w) at the same temperature θ, as follows (Jeantet *et al.*, 2008b):

$$a_w = \frac{P_p}{P_w} \tag{19.2}$$

The RH of air is the ratio between the water vapour pressure of the air (P_a) and the saturation vapour pressure of water (P^0) at the same temperature θ:

$$RH = \frac{P_a}{P^0} \times 100 \tag{19.3}$$

At equilibrium, for a given product, at temperature θ, $P_p = P_a$ and $P_w = P^0$. The a_w or the RH at equilibrium (RH_E) of a

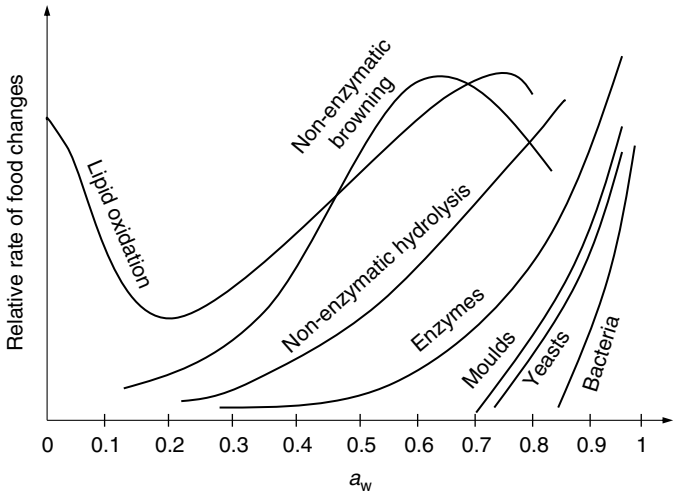


Figure 19.10. Relative kinetics of food spoilage and modification as a function of a_w.

product is equal to the RH of an atmosphere in equilibrium with the product.

$$RH_E = a_w \times 100 \quad (19.4)$$

Thus, measuring the a_w of a product involves determining the RH (using a pressure sensor for example) of a small quantity of air placed in equilibrium with a sample of product to be examined; the amount is such that its water content does not change while reaching equilibrium.

Figure 19.10 shows the changes in the reaction constants (during food modification) as a function of a_w. It shows that in general, product preservation is at a maximum when a_w is 0.2 at 25°C (Efstathiou *et al*., 2002).

Parallel to measuring a_w, it is possible to establish the relationship between a_w and the water content of the product (kg of water per kg of dry matter) at a given temperature θ. This relationship represents the sorption isotherm (adsorption and desorption) as a sigmoidal curve. The sorption isotherms reflect the adsorption capacity of water but also the water retention of products, both of which represent very important parameters for food technology.

Sorption isotherms vary from one food to another. They are the result of the behaviour of various chemical food components with water. Thus, proteins and starches retain more water in the lower region of the isotherms than lipids and crystalline substances (sugar for example). Dried fruits, rich in sugars, are particularly hygroscopic, but only above a certain RH.

The ideal water content for an optimal preservation of a given powder can be determined based on sorption isotherms. Thus, for an a_w of 0.2, the water content of milk powder would be 4% (regulated), between 2% and 3% for whey and 6% (regulated) for casein. Sigmoidal sorption curves can be divided into three sections:

1. The first section (0–0.2 a_w) corresponds to the sorption of a monomolecular layer with strong hydrogen bonds (about 4–60 kJ/mol). This water is bound to polar groups of certain compounds, mainly NH_3^+ and COO^- groups of proteins and OH^- groups of starches; this section also includes water of crystallisation of salts and sugars, that is very strongly bound water, which is relatively difficult to eliminate by dehydration and which is non-freezable.
2. The second section (0.2–0.6 a_w), the linear portion of the curve, corresponds to additional water layers or multimolecular layers with weaker hydrogen bonds (about 1–3 kJ/mol) representing increasingly free water as a_w increases. Changes in the state of carbohydrates (amorphous/crystalline) generally occur in this water activity range (Vuataz, 1988).
3. The third section (above 0.6 a_w) represents condensed water in pores (energy level around 0.3 kJ/mol): this water allows the dissolution of soluble elements (notably mineral salts) and can serve as a support for biological agents such as enzymes and microorganisms (Fig. 19.10). This section is practically asymptotic and therefore very difficult to model.

19.4.1.2.2 Glass transition

Glass transition is a phenomenon whose technological importance has been recognised for many years for mineral and organic substances as well as for food products. This concept, developed and originally used in polymer chemistry, characterises the mobility of water in amorphous

products (i.e. non-crystallised). A distinction is made between (i) products in the glassy state, relatively hard, low water mobility; and (ii) products in the rubbery state (sticky) with greater water mobility and therefore less stable in terms of storage.

The gradual transition from one state to the next is called 'glass transition': it occurs as a result of a variation in temperature or water content. According to Genin and René (1995), the cooling of a pure liquid can, in most cases, lead to a formation of a crystalline solid. This change of state occurs theoretically for a given product at a fixed temperature called the crystallisation temperature (T_c). However, it can happen that during cooling, T_c is bypassed without a change of state. Two cases may then arise if the temperature continues to be lowered:

1. Crystallisation occurs but at a temperature below T_c.
2. The liquid state persists until solidification occurs (without a thermodynamic change of state), at a temperature known as the 'glass transition temperature'.

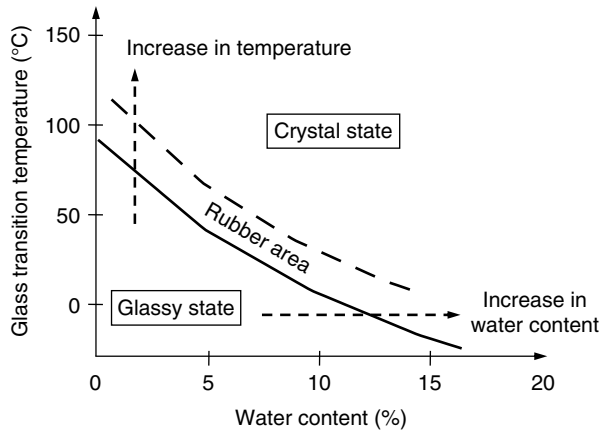


Figure 19.11. Glass transition temperature as a function of water content. Reproduced from Schuck *et al.* (2012), with permission of John Wiley & Sons, Ltd., including further data from Roos (2002).

This rigid liquid is called glass or an amorphous structure. In terms of energy, it is a metastable state: a low energy input will switch it to a more stable state, which can be the liquid or crystalline state.

The glassy state can be achieved in two ways: (i) when cooling is fast enough to avoid the appearance of crystals; and (ii) when dynamic viscosity, by lowering diffusivity (these two parameters are inversely proportional variables), affects the rate of crystal growth.

In the amorphous solid state, molecules are not ordered and the system can be considered to be in a glassy state: the material is characterised by a high internal viscosity. During heating, the system moves from the glassy state to a viscoelastic state in which molecular mobility is higher (Bhargava & Jelen, 1996). This trend can also be obtained at a constant temperature by increasing the water content: this is referred to as plasticising effect. Similarly, the higher the water content, the lower the T_g. This influence of the water content on the T_g was observed for a number of products: amorphous lactose, SMP, etc. (Le Meste & Simatos, 1990; Jouppila & Roos, 1994). Water availability in a food matrix, which determines its stability, is therefore based on many different factors: water content, solute composition, hygroscopicity, viscosity and T_g (Fig. 19.11). For example, the maximum water content generally specified for skimmed milk powder (4% w/w) is defined for optimum stability at 25°C. Under these conditions, the a_w should be close to 0.2 and the T_g close to 50°C (Schuck *et al.*, 2007).

Thus a variation in temperature and water content around the T_g value is accompanied by a considerable change in the mechanical properties of the material (Roos, 1997). Although glass transition has been used to predict the stability of frozen or dried products, the complexity and heterogeneity of the products make interpretations difficult. Sugars, proteins and fat are affected by glass transition (Roos & Karel, 1991a, b). Figure 19.12 summarises the existing relationships between the amorphous and crystalline structures.

Table 19.3 shows some T_g values of components and dried ingredients. We can observe that T_g varies significantly from one component to the next (lactose/casein) and even within the same component category (lactose/galactose). For example, monosaccharides usually have a lower T_g than disaccharides.

19.4.1.3 Protein modifications

Globular proteins can be denatured during different technological operations involving heat transfer (pasteurisation, evaporation, drying). This denaturation can be limited to conformational changes without loss of solubility, which are more or less favourable from a functional point of view. However, it can go as far as irreversible aggregation and gelation, which modifies the quality of the products and encourages fouling of the heat exchange surfaces, resulting in a reduction of heat transfer coefficients. Thus, all soluble milk proteins in whey have denaturation temperatures of between 65 and 75°C; at the pH of milk and an initial protein concentration, the order of heat sensitivity of different milk proteins is immunoglobulin > bovine serum albumin > β-lactoglobulin > α-lactalbumin.

Denaturation of soluble proteins is an indicator of the intensity of the heat treatment and the quality of the

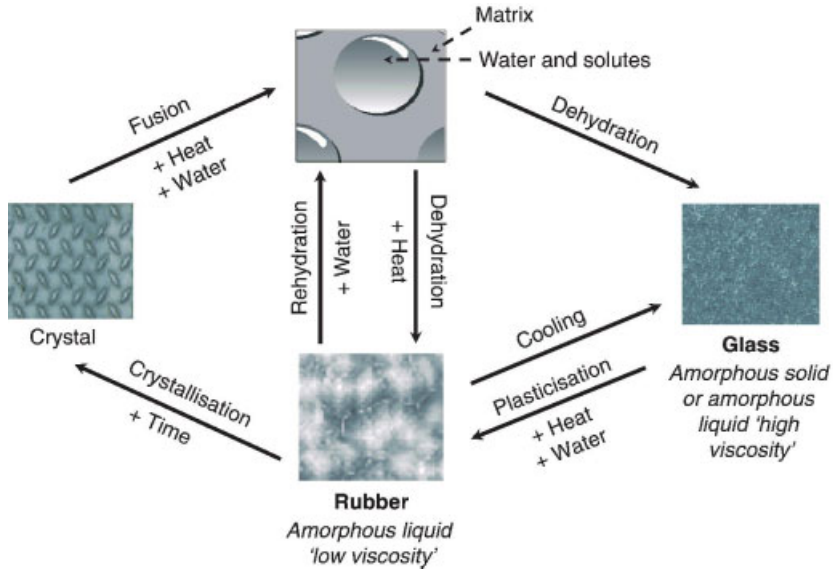


Figure 19.12. Relationships between amorphous and crystallised structures. Reproduced from Schuck *et al.* (2012), with permission of John Wiley & Sons, Ltd., including further data from Roos (2002).

Table 19.3. Glass transition temperatures (T_g) of different carbohydrates and dairy ingredients.

Ingredients	T_g (°C)
Glucose	**36**
Galactose	30
Fructose	10
Sorbitol	–2
Lactulose	–2
Maltose	43
Sucrose	67
Lactose	97
Skimmed milk	92
Whole milk	92
Hydrolysed milk	49
Casein	144
Sodium caseinate	130
Maltodextrin DE 6	168
Maltodextrin DE 33	130
Maltodextrin DE 47	103

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powder, which affects the rehydration properties. The WPNI and the non-casein nitrogen (NCN) are commonly used in the dairy sector to classify powders according to their thermal history. The WPNI is an indirect measure of the intensity of the heat treatment applied to milk during the process of obtaining a powder. Only heat treatment of casein above 90°C for several minutes gives rise to significant changes (Walstra & Jeness, 1984; Le Ray *et al.*, 1998).

19.4.2 Nutritional properties

The French Federation for Nutrition and the Computer Centre on Food Quality (FFN-CIQUAL, 1987) provides the nutritional values of most dairy products. An example is shown in Table 19.4 for SMP.

The nutritional quality of milk powders depends greatly on the intensity of the different heat treatments during the technological process. Heat treatments induce physicochemical changes which tend to reduce the availability of nutrients (vitamin destruction, loss of available lysine content, denaturation of soluble proteins) or to produce nutritionally interesting compounds such as lactulose (Schaafsma, 1989).

19.4.3 Process properties of dairy powder

19.4.3.1 Particle size and powder structure

The size of powder particles, determined by granulometry, is a major characteristic as far as this parameter affects several physical and functional properties (flow, density,

Table 19.4. Nutritional value of skimmed milk powder.

Constituents	Unit	Value
Energy	kJ/kg	14 780.0
Water	g/kg	40.0
Total solids	g/kg	960.0
Proteins (N×6.38)	g/kg	355.0
Total lipids	g/kg	8.0
Available carbohydrates	g/kg	528.0
Vitamins		
Retinol	µg/kg	Traces
β-carotene	µg/kg	Traces
Vitamin D	µg/kg	0.0
Vitamin E	mg/kg	Traces
Vitamin C	mg/kg	50.0
Thiamine	mg/kg	3.8
Riboflavin	mg/kg	18.0
Niacin	mg/kg	10.0
Pantothenic acid	mg/kg	35.0
Vitamin B_6	mg/kg	2.5
Vitamin B_{12}	µg/kg	30.0
Free folic acid	µg/kg	360.0
Total folate	µg/kg	430.0
Biotin	µg/kg	150.0
Mineral salts		
Sodium	mg/kg	6820.0
Magnesium	mg/kg	1120.0
Phosphorus	mg/kg	11 060.0
Potassium	mg/kg	15 370.0
Calcium	mg/kg	13 010.0
Iron	mg/kg	5.2
Copper	mg/kg	2.0
Zinc	mg/kg	47.5
Aminoacids		
Isoleucine	mg/g N	381.0
Leucine	mg/g N	631.0
Lysine	mg/g N	503.0
Methionine	mg/g N	165.0
Cystine	mg/g N	58.0
Phenylalanine	mg/g N	320.0
Tyrosine	mg/g N	304.0
Threonine	mg/g N	297.0
Tryptophan	mg/g N	90.0
Valine	mg/g N	442.0
Arginine	mg/g N	239.0
Histidine	mg/g N	176.0
Alanine	mg/g N	230.0
Aspartic acid	mg/g N	505.0
Glutamic acid	mg/g N	1 386.0
Glycine	mg/g N	138.0
Proline	mg/g N	615.0
Serine	mg/g N	359.0
Aminoacids		
Isoleucine	mg/kg	21.2
Leucine	mg/kg	35.1
Lysine	mg/kg	28.0
Methionine	mg/kg	9.2
Cystine	mg/kg	3.2
Phenylalanine	mg/kg	17.8
Tyrosine	mg/kg	16.9
Threonine	mg/kg	16.5
Tryptophan	mg/kg	5.0
Valine	mg/kg	24.6
Arginine	mg/kg	13.3
Histidine	mg/kg	9.8
Alanine	mg/kg	12.8
Aspartic acid	mg/kg	28.1
Glutamic acid	mg/kg	77.1
Glycine	mg/kg	7.7
Proline	mg/kg	34.2
Serine	mg/kg	20.0

Source: based on data from FFN-CIQUAL (1987).

solubility, wettability, etc.). Particle size is mainly influenced by the droplet size during spraying (Fig. 19.9). It therefore depends on spray conditions and the viscosity of the concentrate; high spray pressure and low viscosity reduce particle size.

19.4.3.2 Flowability–floodability

The ability of a powder to flow freely (i.e. not to form lumps or aggregates) is an important property as regards storage, discharge, weighing, blending, compression, transfer, etc. The study of this property is very complex as is the development of adequate measurement methods and the interpretation of results. The method described by Carr (1965) is used to determine two types of behaviour: flowability and floodability (indices between 0 and 100). Flowability involves measuring the angle of repose, the angle of spatula, cohesion and compressibility. Floodability involves measuring the angle of fall, the angle of difference, distribution in the air and the value of the flow index. Other methods are also used to evaluate powder flow such as measuring the time required for a given volume of powder to flow out of a rotating drum through a given opening (Haugaard Sorensen *et al.*, 1978).

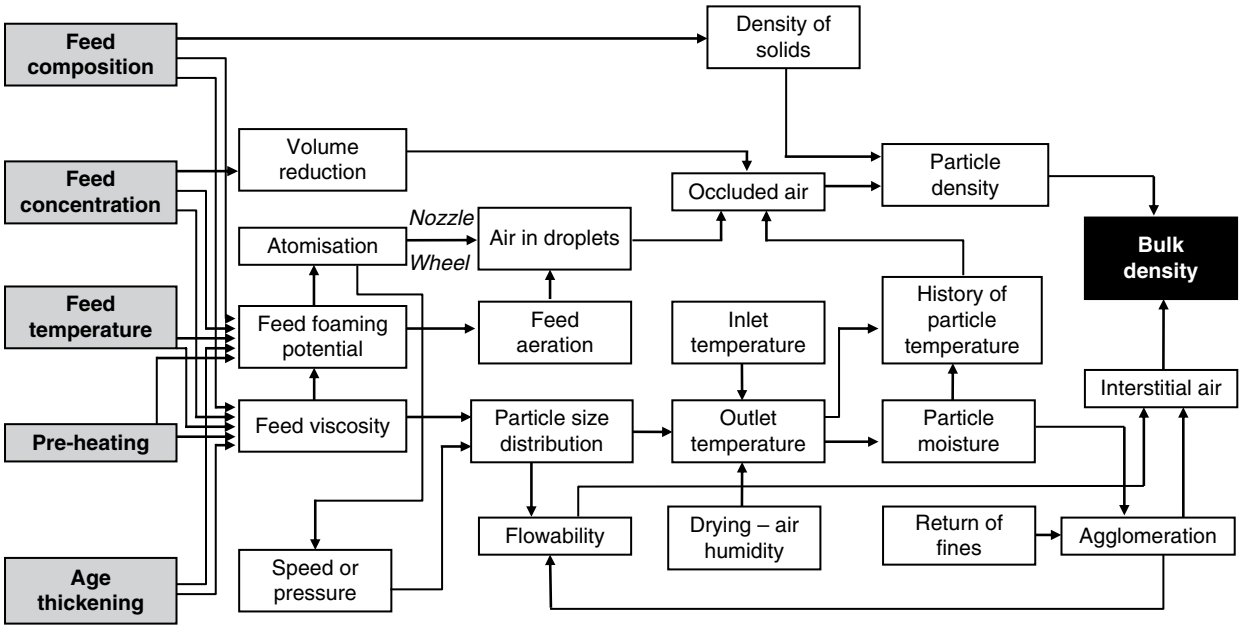


Figure 19.13. Influence of drying parameters and physicochemical characteristics on bulk density. Reproduced from Schuck *et al.* (2012), with permission of John Wiley & Sons, Ltd., including further data from Pisecky (1997) and Masters (2002).

19.4.3.3 Density

Powder density is an important property from an economic, commercial and technological point of view. A high-density powder can reduce mass packaging, transport and storage costs. Density also affects certain functional properties of powders, in particular hydration properties.

The bulk density of a powder is expressed in kg/m^3. It is a complex property that depends on primary factors such as the true or absolute density of a product, the air within each particle (occluded air content) and the air between each particle (interstitial air).

Bulk density is mainly influenced by the characteristics of the concentrate (heat treatment, aeration, foaming capacity, viscosity), those of the drying air (inlet and outlet air temperature) and those of the powder (size distribution of particles and residual moisture) (Fig. 19.13). Bulk density can be increased by adjusting the spray nozzle (influences occluded air) and reducing the uniformity of the particle size distribution (closer packing) by increasing the dry matter content of the concentrate or reducing the spray pressure. In contrast, powders obtained via heated rollers have a low bulk density (300–500 kg/m^3), due to the irregular shape of particles and despite a compact structure without occluded air.

True or absolute density is determined by the chemical composition of the powder and depends on the true density of each component. The level of occluded air depends on technological factors: the agitation speed of the concentrate in the tank, possible foaming of the concentrate, the spray system (a rotary atomiser disc generally leads to a higher occluded air content than nozzle spraying; Table 19.1). The occluded air content is generally determined using a gas pycnometer (air or helium).

Interstitial air depends mainly on the size distribution of particles and the degree of agglomeration (Carić, 2002). It is determined by compacting the powder obtained through successive tapping.

19.4.3.4 Rehydration properties

Most food additives and ingredients are in powdered form and must be rehydrated before use; thus, water–component interactions and, more generally, the suitability for reconstitution of a powder in water are the main properties in the development and formulation of these food products (Hardy *et al.*, 2002). These properties depend on the one hand on the composition of the powder and in particular the affinity between components and water, and on the other hand the steric accessibility of water (porosity and capillarity) to powder components.

Rehydration properties include all the stages that lead to the total dissolution of the powder. These are as follows:

- *Wettability* refers to the surface hydration of particles. Sinkability is often associated with this stage, which refers to the capacity of the particles to overcome the surface tension of the solvent. A powder is considered wettable if its wettability index is less than 120 s (Haugaard Sorensen *et al*., 1978).
- *Dispersibility* corresponds to the ability of a powder not to form aggregates (lumps) when placed in solution. For example, milk powder is dispersible if its dispersibility index is greater than 90% for skimmed milk and greater than 85% for whole milk. However, with improvements in spray drying technology, milk powder is now considered to be dispersible if its dispersibility index is greater than 95% (Haugaard Sorensen *et al*., 1978).
- *Solubility* corresponds to the disappearance of the granular structure after complete solubilisation of the powder. For example, milk powder is soluble if its solubility index is greater than 99% or if its insoluble content is less than 1% (Haugaard Sorensen *et al*., 1978). A distinction is made between real insoluble material, linked to the thermal denaturation of components, and apparent insoluble material, linked to the incomplete insolubilisation of a product in terms of its biochemical composition and rehydration kinetics (Schuck *et al*., 1994).

These different phenomena occur consecutively or simultaneously during rehydration and influence each other, which makes it difficult to quantify them individually. Rehydration properties are evaluated using three reconstitution indices: wettability, dispersibility and solubility. Hygroscopicity is often added to these indices, completing the description of water (including vapour form)/particle interactions.

19.4.3.5 Hygroscopicity

The hygroscopicity of a powder is defined as its final moisture content after being introduced to air at a controlled RH, and measured according to the Westergaard (2004) method. It can therefore be deduced from sorption isotherms. A powder is considered non-hygroscopic if the percentage of hygroscopicity is less than 10%.

19.4.3.6 Instant powders

It is generally accepted that under predefined reconstitution conditions, a milk powder can be considered instant if its wettability is less than 20 s, its dispersibility is greater than 95% and its solubility is greater than 99.0%. In actual fact, in most cases a solubility greater than 99.5% or even 99.7% and a dispersibility greater than 98% are required (Schuck *et al*., 2012).

Most standards are defined to characterise skimmed milk or WMPs. However, the diversification of industrial powdered products is such that these three indices in a powder can be measured under the same reconstitution conditions as the end user (dry matter, temperature, agitation, etc.).

Dispersibility is probably the best sole criterion to evaluate the 'instantaneous' character of a powder because to some extent it is correlated with other rehydration properties (solubility and wettability).

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20
Frozen Dairy Foods

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20.1 INTRODUCTION

Despite the myths surrounding the origins of ice cream, we do not know for sure how the evolution of ice cream resulted. Snow and ice have been popular during warmer seasons because of the cooling properties of these materials. Perhaps first snow and ice were mixed with fruit juices and later with milk or yogurt and this resulted in a gradual evolution of these products as we know them. Until the nineteenth century, harvesting ice and storing it for use during summer was a labor-intensive process and therefore ice cream was a food for the rich only. With the invention of the hand-cranked freezer and a ready availability of ice, ice cream moved down the social ladder, and towards the end of the nineteenth century it was sold on the streets of metropolitan areas (Visser, 1986).

20.2 TECHNOLOGY ESSENTIALS

Ice cream is manufactured as regular, custard/French, reduced fat, light, and low- and no-fat versions. Other frozen desserts include frozen yogurt, sherbet, water ice, gelato, mellorine, frozen dairy dessert, frozen confection, frozen dairy confection, milk shake, smoothies, shake, and slush. The nomenclature varies from country to country depending on the prevailing legislation. Two manufacturing practices that affect the characteristics of frozen desserts are the freezing technique and degree of freezing. The freezing technique may involve stirring (agitation) during freezing, or without stirring (quiescent), or a combination of the two. The degree of freezing results in products that are hard frozen, or designed for dipping or scooping, or used as soft serve or milk shakes (Kilara, 1992).

20.2.1 Classification of and trends in the frozen desserts market

The chemical composition of ice cream differs mainly with regard to the fat content, which is required by law, and three grades of ice cream can usually be found in most market areas. One grade (economy) just meets the minimum fat content, often has an overrun (a measure of air incorporated) that approaches the maximum allowed by law, and usually contains relatively inexpensive flavor ingredients. At the other extreme are the so-called premium ice creams that are high in fat, low in overrun, and usually contain natural flavors. A third grade of ice cream (regular), designed as a compromise between the minimum cost and premium products, is the type that has dominated the market for many years. Newer developments have introduced a fourth grade referred to as super premium ice cream that is characterized by higher fat contents and lower overruns than premium varieties. Cost of the product is directly proportional to the grade of the ice cream (Kilara, 1992).

The volumes of ice cream from cow milk and other frozen dairy desserts produced in the USA in 2010 are shown in Table 20.1 (USDA, 2011). The frozen dessert market is predominantly the ice cream market. In 2009, the ice cream market amounted to 1.52 billion gallons (5.75 billion L). It constituted 86.7% of the total volume of all frozen desserts. The rest of the market primarily consists of frozen yogurt, water/fruit ices, and sherbet. Regular ice cream represented 60.5% of the total hard and soft ice cream market, whereas low-fat and non-fat ice cream represented 26.2%. Per-capita consumption of regular ice

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Table 20.1. Production statistics of various frozen desserts from cow milk and non-milk desserts in the USA in 2009 and 2010.

	Million US gallons produced (million liters in parentheses)	
Frozen dessert	**2009**	**2010**
Ice cream, regular, total	918.238 (3475.91)	912.369 (3453.69)
Ice cream, low fat, total	399.667 (1512.90)	380.030 (1438.57)
Ice cream, non-fat, total	16.771 (63.49)	16.176 (61.23)
Frozen yogurt, total	46.026 (174.23)	49.740 (188.29)
Sherbet, total	53.277 (201.68)	49.272 (186.51)
Water and juice ices, total	60.130 (227.62)	61.228 (231.77)
Other frozen desserts, total	15.255 (57.75)	12.254 (46.39)

Source: USDA (2011).

Table 20.2. Comparative composition (%) of various frozen desserts.*

	Ice cream					
Constituent	**Non-fat**	**Low fat**	**Reduced fat**	**Sherbet**	**Water ice**	**Frozen yogurt**
Milk fat	0.5	3.0	6.0	1.5	0	2.16
Milk solids-not-fat	13.5	13.0	12.5	3.5	0	10.11
Sucrose	10.0	9.0	10.0	23.0	23.0	12.6
Corn syrup solids 34/42 DE†	10.0	9.0	8.0	7.0	7.0	5.4
Maltodextrins 10 DE	0	0	0	0	0	3.6
Whey protein conc. 34%	0	0	0	0	0	2.4
Stabilizer/emulsifier	1.0	0.8	0.6	0.4	0.4	0.6
Total solids	35.0	34.8	37.1	35.4	30.4	36.87

*Blended in the mix are flavorings, cocoa, nuts, fruits, bakery items, and other food materials before/during freezing process.

†DE is dextrose equivalent; indicates degree of hydrolysis of starch.

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cream was approximately 3 gallons (11.36 L), whereas that of the low-fat and non-fat category was approximately 1.3 gallons (4.9 L). The popularity of flavors in descending order was vanilla (27.8%), chocolate (14.3%), strawberry (3.3%), chocolate chip (3.3%), and butter pecan (2.8%) (Schultz, 2011).

20.2.2 Formulation

To make an ice cream mix, three categories of ingredients are necessary. A concentrated source of milk fat is the first category, the second is a concentrated source of milk solids-not-fat (MSNF) (also known as serum solids), and the third is balancing ingredients. Composition of various frozen desserts is shown in Table 20.2 (Kilara & Chandan, 2008). Representative formulae for commercial grades of ice cream are shown in Table 20.3.

Regarding frozen yogurt, there are no Federal standards in the USA. The product may be defined as a food prepared by freezing while stirring a blend of pasteurized non-fat or low-fat ice cream mix and yogurt (Marshall & Arbuckle, 1996). Yogurt used for blending with ice milk mix must comply with the federal and state compositional standards for yogurt. It must be cultured with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to titratable acidity, a minimum of 0.85%. In general, frozen yogurt mix obtained by blending yogurt and low-fat/non-fat ice cream has a pH of 6.0 or titratable acidity of 0.30%. Thus, the industry standards require minimum titratable acidity of 0.30%, with a contribution of approximately 0.15% as a consequence of fermentation by yogurt bacteria. Most manufacturers use 10% of yogurt in their formulations. As a consequence, frozen yogurt tastes very similar to

Table 20.3. Representative formulae showing percentage composition for ice cream mixes of different grades.*

Component	Non-fat	Low fat	Reduced fat	Regular	Premium	Super premium
Milk fat	0.50	3.0	6.0	10.0	12.0	16.0
Milk solids-not-fat	13.5	13.0	12.5	8.5	9.5	9.0
Whey solids	—	—	2.0	2.0	—	—
Sugar	10.0	9.0	10.0	11.0	11.0	16.0
Corn syrup solids	10.0	9.0	8.0	6.5	6.0	—
Stabilizer/emulsifier	1.0	0.8	0.6	0.4	0.3	—
Total solids	35.0	34.8	39.1	38.4	38.8	43.0
Water	65.0	65.2	60.9	61.6	61.2	57.0

*Blended in the mix are flavorings, cocoa, nuts, fruits, bakery items, and other food materials before/during freezing process.

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Table 20.4. A typical formulation of low-fat frozen yogurt.

Component	Skim milk yogurt: usage level 10%	Ice milk: usage level 90%	Frozen yogurt mix (blend of yogurt and ice milk)
Milk fat (%)	0.07	2.39	2.16
Milk solids-not-fat (%)	10.96	10.02	10.11
Whey protein conc. 34% (%)	0.0	2.7	2.4
Sucrose (%)	4.0	13.5	12.6
Corn syrup solids 36/42 DE (%)	0.0	6.0	5.4
Maltodextrins 10 DE (%)	0.9	4.0	3.6
Stabilizer (%)	0.0	0.7	0.6
Total solids (%)	15.03	36.04	36.87
% titratable acidity	0.85	0.15	0.30
pH	4.6	6.7	6.0

Source: reproduced from Chandan & O'Rell (2006), with permission of Blackwell Publishing.

low-fat/non-fat ice cream, with a hint of yogurt flavor at the end. This flavor attribute is preferred by the consumer in that the perceived health attributes of yogurt bacteria are available along with the popular taste of low-fat/non-fat ice cream.

A typical formulation of low-fat frozen yogurt is given in Table 20.4. The table shows a mix composed of 10% sweetened plain non-fat yogurt and 90% low-fat ice cream mix. If a lower pH (<6.0) is desired in the finished product, the proportion of plain yogurt can be increased to more than 10% and vice versa.

20.2.2.1 Concentrated sources of milk fat

Fat in milk is secreted as tiny droplets called globules. A drop of milk contains millions of such globules. Each globule is surrounded by a milk fat globule membrane, which is made up of triglycerides, traces of diglycerides and monoglycerides, cholesterol, phospholipids, and many other substances. The triglycerides, which are the main components, are synthesized by the cow by linking three molecules of fatty acids to one molecule of glycerol. Fatty acids can have as few as four or as many as 26 carbon atoms. Fatty acids containing four, six, eight, or 10 carbon atoms are significant because milk fat flavor is in large part due to the presence of these short-chain fatty acids: butyric, caproic, caprylic and capric acids, carbon numbers 4, 6, 8, and 10, respectively. Further fatty acids are either saturated or unsaturated with one double bond (monoenoic), two double bonds (dienoic), or three (trienoic). The unsaturated fatty acids with multiple double bonds are valuable functional, healthy, and essential foods in human nutrition (Kurtz, 1974).

Milk fat melts and crystallizes, is unctuous, depresses the cold sensation, contributes desirable flavor, is a solvent

Table 20.5. Role and sources of various components of ice cream.

	Role and functions	Limitation	Sources in order of preference
Milk fat	Imparts desirable creamy rich flavor Source of fat-soluble vitamins Improves body texture Improves melting resistance	Calorie dense with excessive fat (17%) gives too much viscosity to mix and hinders whipability Source of oxidized, rancid, and fishy flavor defects	Fresh sweet cream Fresh milk Frozen cream
Milk solids-not-fat	Improves texture Imparts better body Source of protein, minerals, and vitamins	Improper levels cause "sandiness" defect Source of desirable cooked flavor	Dry milk Fluid whole milk Fluid skim milk Condensed skim milk Skim milk powder Whey products
Sweeteners	Impart sweet flavor	Too much sweetener, especially corn syrup, impedes freezing process	Cane sugar, corn syrup solids, high fructose corn syrup

Source: reproduced from Kilara & Chandan (2008), with permission of John Wiley & Sons.

for added flavors, adds structure to ice cream, and is of great importance in extrusion properties of ice cream. Extrusion helps shape ice cream into novelties. Milk and cream constitute the most important components of ice cream, because they furnish the basic ingredients for a good-quality product. Other ingredients provide flavor, body, and texture of the frozen dessert (Kilara & Keeney, 1989).

The nature and intensity of ice cream flavor is a function of the flavor quality of the individual constituents and subsequent processing treatment of the ice cream mix. Flavor defects in ingredients cannot be alleviated during ice cream making. Flavor problems can be compounded as a consequence of negligent processing procedures.

The body or consistency of ice cream is related to the mechanical strength of the mix and its resistance to melting. Heat shock resistance is dependent on the nature and concentration of the stabilizer–emulsifier system used. The texture of ice cream depends upon the size, shape, number, and arrangement of fat globules, ice crystals, and the ratio of liquid and frozen water in the ice cream (Kilara & Keeney, 1989).

Balancing quality and cost is a major challenge to the frozen dessert manufacturer. Satisfactory composition produces ice cream with an optimum combination of cost, flavor, body, and texture, cooling effect, viscosity, whipping ability, and freezing characteristics. Factors responsible for overall ice cream quality are raw material quality, sanitary care during mix preparation, processing parameters, flavoring used, freezing techniques, and storage conditions.

Formulation of frozen dessert mix involves utilization of both the fat and the solids-not-fat components of milk. The functions and preferred sources of major ice cream ingredients are summarized in Table 20.5 (Kilara & Chandan, 2008).

Whole milk may be used primarily as a source of milk solids. There is no better source of fat than sweet cream because of its desirable flavor, convenience of handling, and good whipping characteristics. Fresh cream is judged by flavor, acidity, and bacterial count. The titratable acidity should be low and show no evidence of developed acidity. When fresh cream is not available at a favorable cost, alternative sources of fat should be considered. The high price of sweet cream during certain seasons of the year makes storage of cream during the months of surplus economically attractive. All known precautions must be used to ensure prevention of the development of off-flavors in stored cream. Only the best cream should be processed for storage, and it should contain no developed acidity. Off-flavors likely to develop in frozen cream are rancid, fishy, oily, and tallowy. Hydrolytic rancidity is due to free butyric acid from the partial hydrolysis of milk fat brought about by enzymatic activity of lipase on fat or by enzymes of certain bacteria. A proper heat treatment regimen, like pasteurization, is an essential phase for the preparation of cream for freezing, and consists of heating cream at 76.7°C for 20 min, or 82.2°C for 10 min, or 87.8°C for 5 min. This treatment not only inactivates

the lipase enzyme naturally present in milk but also destroys 95–99% of the bacteria present (Kilara & Chandan, 2008).

Heat treatment of cream also increases the resistance of the cream to oxidation. A fishy flavor in dairy products results from the formation of trimethylamines by the hydrolysis and oxidation of lecithin, a naturally occurring phospholipid in milk. Factors that promote development of this off-flavor are high acidity and the presence of prooxidants (iron or copper salts). Heat treatment at the above times and temperatures "activates" or uncoils the proteins so that sulfhydryl groups are exposed and become oxidized by atmospheric oxygen in preference to the unsaturated fatty acids. These sulfhydryl groups function as antioxidants in the liquid phase and may complex with prooxidant minerals (Kilara & Chandan, 2008).

Following heat processing, the cream is quickly frozen. Proper packaging and handling of frozen cream are also important. Preferred packages include stainless steel or plastic containers. Quick-frozen cream is held at –20°C. Disadvantages of frozen cream include the necessity of thawing before use and the fact that it is messy to handle.

Owing to fluctuating supplies and the price of cream and the disadvantages of frozen cream, some manufacturers rely on unsalted sweet cream butter. Butter is manufactured by agitating or churning cream. Cream is an oil-in-water emulsion. The aqueous phase is skim milk. Agitation of cream results in a phase inversion converting oil-in-water emulsion to a water-in-oil emulsion. The serum is separated as buttermilk. This buttermilk is not to be confused with cultured commercial buttermilk, which is a different product. Buttermilk is dried into powder and can be used as a source of serum solids in ice cream formulations. Unsalted butter is packaged in 25-kg (56-lb) blocks. Butter for immediate use should be stored refrigerated. For extended shelf-life, butter should be stored frozen. To use butter as a concentrated source of milk fat requires melting and is considered a processing inconvenience (Kilara & Chandan, 2008).

In some parts of the world where refrigerated storage is at a premium, butteroil may be used by ice cream manufacturers as a concentrated source of milk fat. Butteroil is manufactured by heat treatment, removing moisture and residual serum solids from unsalted butter. Butteroil is sold in 206.25-L (55-gallon) drums and is stored under ambient conditions. During storage, milk fat crystallizes and this may necessitate the warming of the oil prior to use in mix making. Butteroil is packed under nitrogen to delay the onset of rancidity.

The preferred source of concentrated milk fat (from most to least) is cream, unsalted butter, and butteroil. Choice depends upon availability, economics, local preferences, regulatory factors, and quality of the ingredients (Kilara & Chandan, 2008).

20.2.2.2 Concentrated sources of serum solids

MSNF is skim milk solids and these are made up of lactose, protein, and milk salts. Proteins play an important role in emulsification of the fat. Milk proteins also help in developing overrun (aeration). The combination of emulsification and foaming create desirable texture. Proteins also contribute to the viscosity of the mix. Proteins are surface active agents (surfactants), and are responsible for desirable interfacial behavior (Kilara & Keeney, 1989).

Fluid whole and skim milk are excellent sources and should be used in the ice cream mix. However, because of their low solids content in contrast to the solids desired in ice cream mix, their use is limited.

Fresh condensed skim milk is easy and convenient to use and has an excellent flavor. Method of payment for concentrates is based on total solids content. The total solids content is usually around 25–30%. The heat treatment given fluid skim milk is usually the same as the regular pasteurizing range. The keeping quality of condensed skim milk is better than that of cream. It should be stored at 0–1°C and used while fresh and sweet (usually for 7–10 days). Plain condensed whole milk is concentrated about two and a half times and contains 8% fat and 20% serum solids. The use of superheated condensed milk may substitute for the use of heat concentrated milk. The already-condensed product is slowly heated to a high temperature, usually around 82.2°C. When properly done, a concentrate of much greater viscosity is obtained, which improves the whipping ability of the ice cream mix and contributes a smooth texture, which then binds more free water. Accordingly less water is available to form ice crystals during freezing and shelf-life, and the smooth texture of the ice cream is maintained throughout its shelf-life. Superheating therefore functions like a stabilizer (Kilara & Chandan, 2008).

Sweetened condensed whole or skim milk may sometimes be used as a source of MSNF. This ingredient provides 8.5% fat and 28% total milk solids. The added sugar (40–44%) improves the keeping quality over that of plain condensed milk. With this concentration of sugar, the osmotic pressure of the solution is high enough to suppress the growth of practically all microorganisms. The product will keep at room temperature.

The titratable acidity test should be applied to all condensed milk products. When diluted so as to contain the same MSNF concentration as skim milk, the acidity should be approximately that of fresh skim milk (0.18%) (Kilara & Chandan, 2008).

Table 20.6. Comparison of properties of nutritive sweeteners.

Sweetener	Relative sweetness	Solubility (g/100 g) at 25°C	Chemical type
Sucrose	1.0	67	Disaccharide
Glucose	0.6	51	Monosaccharide
Fructose	1.2–1.8	81	Monosaccharide
Invert sugar	1.0	—	Glucose and fructose
Lactose	0.3	16	Disaccharide
Sorbitol	0.6	72	Sugar alcohol
Mannitol	0.7	18	Sugar alcohol
Xylitol	1.0	64	Sugar alcohol
Corn syrup solids (36 DE)*	0.45	70	Mixtures
Corn syrup solids (42 DE)	0.45	70	Mixtures
High fructose corn syrup	1.2	67	Mixture

*DE, dextrose equivalent, an indication of the extent of starch hydrolysis. 100 DE represents full hydrolysis of starch to glucose.

Source: reproduced from Kilara & Chandon (2008), with permission of John Wiley & Sons.

20.2.2.3 Balancing ingredients

In order to balance a formula and make a mix, ingredients such as milk, skim milk, or water may be necessary. This is because a concentrated source of milk fat such as cream will contribute serum solids along with the fat. Similarly concentrated sources of MSNF may also contribute fat to the mix, for example condensed whole milk. In instances where liquid sugar is used, water in the ingredient may dilute the solids. Therefore, a balancing ingredient is necessary (Kilara & Chandan, 2008).

20.2.2.4 Sweeteners

The sweetness of ice cream is due to the presence of sugars and other sweeteners. Nutritive sweeteners provide 4 calories per gram of the sweetener and include sugar (sucrose, saccharose), lactose (milk sugar), dextrose (glucose), fructose (fruit sugar, levulose), corn syrup solids (glucose syrup solids), high-fructose corn syrups, and sugar alcohols (xylitol, maltitol, sorbitol, and glycerol). A comparison of the properties of nutritive sweeteners is given in Table 20.6 (Kilara & Chandan, 2008).

Sugar (sucrose) provides sweetness, depresses the freezing point, affects freezing performance, affects body and texture, enhances flavor, and contributes bulk or total solids, and impacts on economics. Generally the equivalent of 15% sucrose is considered optimal sweetness in ice cream. In making no-sugar-added ice cream and frozen desserts, the bulk contributed by sugar is absent and therefore bulking agents such as polydextrose are used. Sugars depress the freezing point of the ice cream mix. Liquid sugar is sugar syrup containing 67% sugar and 33% water. It is used by high-volume ice cream manufacturers and is sold in rail tank cars or truckload quantities. It can be easily pumped and metered into ice cream mix-making operations (Kilara & Chandan, 2008).

Glucose and fructose are simple sugars called monosaccharides. They depress the freezing point of water to a greater extent than disaccharides (sucrose, lactose). They are added as a part of the high-fructose corn syrup mixture. High-fructose corn syrup contains 45% fructose and 55% glucose and has the same sweetness as sugar. By further refining the proportion of fructose can be increased to 55% fructose, with 45% glucose. The resulting product, called high-fructose corn syrup 55, is slightly sweeter than sugar. An additional refining step can increase the fructose content to 90%. This product is called high-fructose corn syrup 90 and is approximately 1.8 times sweeter than sugar.

Sugar alcohols such as sorbitol and xylitol are used in frozen desserts for diabetics. They depress the freezing point to a greater extent than disaccharides and similar to monosaccharides. Glycerol depresses the freezing point to a greater extent than sugar. Alcohols depress the freezing point to an extent greater than glycerol (Table 20.7) (Kilara & Chandan, 2008).

Often corn syrup solids or maltodextrins are added to ice cream mix formulations. Corn sweeteners are derived from the modification of corn starch. Low-conversion corn syrups are 23–38 dextrose equivalent (DE). DE is a measure of the extent of hydrolysis or modification of starch. Regular conversion syrups are 23–38 DE, intermediate conversion syrups

Table 20.7. Effects of nutritive sweeteners on freezing point depression.

Sweetener	Relative effect
Sucrose	1.0
Lactose	1.0
Dextrose	1.82
Fructose	1.82
55% High-fructose corn syrup	1.85
Sorbitol	1.90
Glycerol	3.70
Alcohol	7.40

Source: reproduced from Kilara & Chandon (2008), with permission of John Wiley & Sons.

are 48–58 DE, and high conversion syrups are 58–68 DE. These products are not as sweet as sugar but they contribute total solids to the mix. By increasing total solids to the mix heat shock protection is provided. In some formulations, honey is used as a sweetener. Honey is like invert sugars. It is made up of glucose and fructose. Therefore honey depresses the freezing point to a greater extent than sugar at equivalent concentrations. In certain low-fat and non-fat mixes as well as no-sugar-added mixes, maltodextrins of 5 or 10 DE are used to provide solids in the mix without adversely affecting the freeing point of the mix (Kilara & Chandan, 2008).

Non-nutritive sweeteners are utilized in no-sugar-added ice cream and frozen desserts in which no sugars are added to achieve sweetness. Such formulations rely on the addition of intense non-nutritive sweeteners. The intense sweeteners that do not provide any significant calories at use levels include sucralose, aspartame, saccharin, cyclamates, and acesulfame-K. Natural high-intensity sweeteners are derived from *Stevia* extracts. As little as 0.07% of aspartame provides the sweetness equivalent to 15% sugar. The mass of the formulation is reduced by 14.93%, resulting in more water that must be controlled. To make up this difference, bulking agents are used. One common bulking agent is polydextrose. Additionally, removal of sugar increases the freezing point of the mix. Therefore, to lower the freezing point sugar alcohols are used. A third adjustment necessary for no-sugar-added formulations is the increased level of stabilizers in the formulation. A comparison of the non-nutritive sweeteners is provided in Table 20.8 (Kilara & Chandan, 2008).

A typical formula for a no-sugar-added low-fat ice cream would contain 3% fat, 12% MSNF, 8.0% polydextrose, 5% 10 DE maltodextrin, 1.2% microcrystalline cellulose, 0.35% stabilizer and emulsifier, 0.07% aspartame, and 2.0% sorbitol. The total solids would be 36.62%. This mix would freeze at 2.7°C (27°F) (Kilara & Chandan, 2008).

Table 20.8. Comparisons among high-intensity (non-nutritive) sweeteners.

Sweetener	Relative sweetness	Solubility (g/100 g) at 25°C
Saccharin	250–550	125
Cyclamate	30–50	Not known
Aspartame	120–200	1
Acesulfame-K	100–130	27
Alitame	2000	17
L-Sugars	1.0	67
Sucralose	500–700	30
Stevia extracts	300–350	Very soluble

Source: reproduced from Kilara & Chandon (2008), with permission of John Wiley & Sons.

20.2.2.5 Stabilizers

The term "stabilizer" is used for a group of substances that help stabilize the structure of ice cream. Other names include colloids, hydrocolloids, and gums, which indicate that these substances are large molecules (macromolecules) that are capable of interacting with water. Interacting with water also lets some of these compounds interact with proteins and lipids in the mix. A variety of materials are used as stabilizers. These include gelatin, guar gum, sodium carboxymethylcellulose, microcrystalline cellulose, locust bean gum (carob), and carrageenan. During mix processing, the presence of gums affects mix viscosity and homogeneity, during freezing gums exert secondary effects in dryness and stiffness of ice cream, and in the finished frozen desserts control the properties of the water that is unfrozen. This last point means that ice cream is smoother and ice crystals take longer to grow in the presence of stabilizers especially during storage and distribution of these products.

Usually, stabilizers are used at 0.1–0.5% levels in the mix but the actual amount depends on the type of stabilizer, strength of the stabilizer, total solids and fat level of the mix, duration and temperature of storage of ice cream, and the method of pasteurization. High-fat and high total solids mixes require lesser levels of stabilizers. More stabilizer is needed for ice cream that is stored for a long period of time or if the temperature fluctuation during storage is frequent. If the mix is pasteurized by the high-temperature short-time (HTST) method, more stabilizers may be needed than if the same mix were pasteurized by the batch method or by the ultra-high temperature method (Kilara & Chandan, 2008).

A good stabilizer should be non-toxic, readily disperse in the mix, not cause excessive viscosity, separation or

foam in the mix, not clog strainers and filters, provide ice cream with good meltdown, be economical, and not impart off-flavor to the mix.

Gelatin is an animal protein derivative and is effective at high concentrations of 0.3–0.5% but is expensive and therefore rarely used in the USA. It may not prevent the effects of heat shock. It is also not acceptable to certain religious and vegetarian segments of the population. If gelatin is used as a stabilizer, a long aging period for the mix is necessary. Gelatin disperses easily and does not cause wheying off or foaming (Kilara & Chandan, 2008).

Guar gum is derived from the seeds of a tropical legume called guar. It is the least expensive of the stabilizers and effectively mitigates the undesirable changes in ice cream due to heat shock. It readily disperses in the mix and does not cause excessive viscosity in the mix. Typically 0.1–0.2% is required in a mix and therefore this substance is considered to be a strong stabilizer.

Sodium carboxymethylcellulose is a chemical derivative of cellulose. If used alone, it causes mix separation and therefore it is often blended with carrageenan to prevent wheying off. It is a strong stabilizer. Only 0.1–0.2% is needed in a mix. It imparts body and chewiness to ice cream.

Locust bean gum is also derived from a plant seed and is also known as carob seed gum. It is a strong stabilizer and is used at 0.1–0.2% levels. Mix containing locust bean gum separates or wheys off during storage. It also does not fully hydrate in high temperature short time pasteurized mixes.

Carrageenan is derived from a seaweed *Chondritis crispus*. It is used in many stabilizer blends at levels of 0.01–0.02%. This stabilizer reacts with milk proteins and thereby prevents wheying off in mixes (Kilara & Chandan, 2008).

20.2.2.6 Emulsifiers

As opposed to stabilizers, emulsifiers exert their action on the fat phase of ice cream. Emulsifiers are surface active agents (surfactant). Emulsifiers facilitate the mixing of fat and water because these molecules have two domains, one that likes water (hydrophilic) and another that likes fat (hydrophobic). When the hydrophobic part of a surfactant interacts with the fat, the water-loving part of the molecule can interact with water, thus facilitating the suspension of fat in water. Generally monoglycerides and diglycerides and ethoxylated esters of sorbitol (polysorbates) are the commonly used emulsifiers. Monoglycerides and diglycerides are derived from fatty acids and glycerol. Therefore, emulsifiers are fatty substances. They also show fat-like properties of melting point and crystallinity, and they can be composed of saturated or unsaturated fatty acids. The presence of emulsifiers in ice cream leads to smoother texture and better shape retention, while improving the ability of the mix to incorporate air (Kilara & Keeney, 1989).

Monoglyceride and diglyceride mixtures are obtained by the chemical treatment of fats such as lard, palm kernel, or soybean oil. Most of the monoglycerides and diglycerides are solid at room temperature and are added at a level of 0.1–0.2% to the mix prior to pasteurization. Emulsifiers with high-monoglyceride content are also effective drying agents (Kilara & Chandan, 2008).

Polysorbates are polyoxyethylene compounds. These synthetic chemicals are the most effective drying agents. Polysorbate 80 is an oleic acid derivative. It is a very powerful drying agent and is used at 0.04–0.07% levels. Polysorbate 65 is helpful as a whipping agent, i.e., it helps in air incorporation. To obtain comparable stiffness, more polysorbate 65 has to be used than polysorbate 80. At high levels, polysorbate 80 imparts off-flavors whereas polysorbate 65 does not. Polysorbates are generally liquids and cause churning of the mix (Kilara & Chandan, 2008).

Dried or frozen egg yolks are used to produce dry, stiff ice cream. Dried egg yolks are harder to incorporate into a mix than frozen and sugared egg yolks. The general use level of egg yolks is 0.3–0.5%. If a French-style or custard is required, a minimum of 1.4% egg yolk solids is necessary. Lecithin, a phospholipid present in egg yolks, is thought to act as the emulsifier. Lecithin can also be derived from soybean oil. Buttermilk powder provides phospholipids which can act as emulsifiers (Kilara & Keeney, 1989).

20.2.3 Processing

Figure 20.1 shows various steps involved in the manufacture of ice cream. Knowing a mix specification, mix calculations are performed to determine the amounts of desired ingredients needed to formulate the mix. Many software programs are available to determine the quantity of each ingredient in the formulation of the mix. Mix processing begins with the assembly of the necessary ingredients in the desired amounts. Generally this assembly requires weighing of the ingredients; if liquid ingredients are used, they are metered. Meters rely on knowing the density or the specific gravity of the ingredient, and these values are highly temperature dependent. In most small-scale operations, weighing is the method of choice.

20.2.3.1 Blending

The next step is blending the ingredients together. Mix blending (batching) can be performed at refrigeration temperatures (4°C, 40°F) or at warmer temperatures 45°C (113°F). Cold batching is the preferred method when cream, liquid milk, condensed skim are the ingredients. Warmer temperatures are generally used when the ingredients include butter, butteroil/anhydrous milk fat in combination with non-fat dry milk. Batching begins by placing a

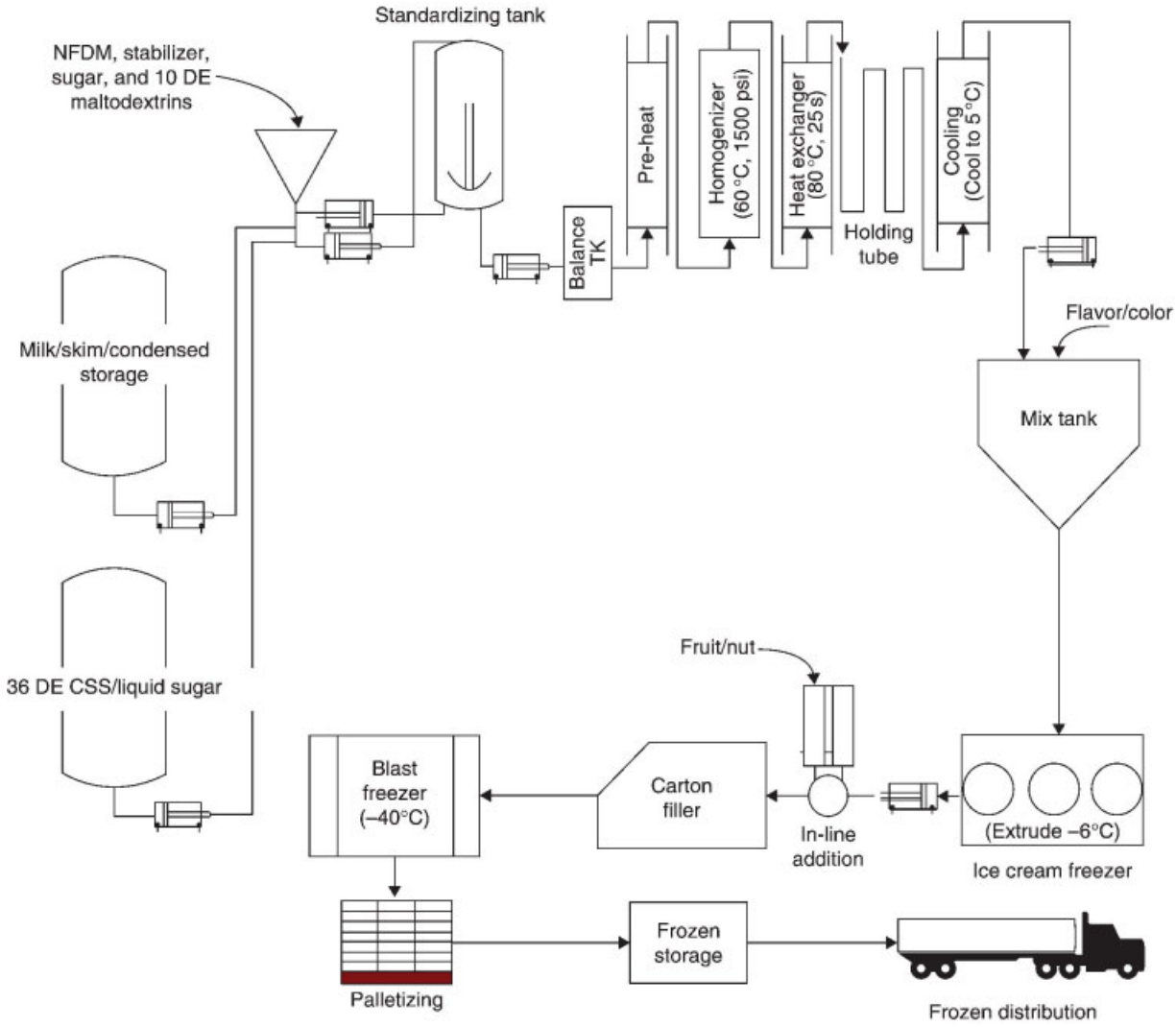


Figure 20.1. Flow sheet for the manufacture of ice cream. NFDM, non-fat dry milk; CSS, corn syrup solids. Reproduced from Kilara & Chandan (2008), with permission of John Wiley & Sons.

liquid ingredient in a vat. Generally this ingredient is skim milk (balancing ingredient) or water. The dry ingredients such as corn syrup solids, maltodextrins, sugar, stabilizers, and emulsifiers are incorporated into this liquid and finally cream is added. When cream is subjected to excessive shear it can churn to butter. This should be avoided. Incorporation of the dry ingredients is aided by one of two types of device: (i) powder horn and (ii) high shear mixers. The powder horn is the easiest device and consists of a funnel with a valve placed in line with a pump and is capable of recirculating the liquid in the vat (Kilara & Chandan, 2008).

With the valve closed, the dry ingredient is placed in the funnel, the pump is started and once the fluid is in recirculation, the valve under the funnel is opened. As the liquid flows past the funnel it sucks in the powder. The mixture of the powder and the liquid hits the impeller of the centrifugal pump, which facilitates the dispersion of the powders. The dispersed powder enters the vat and is recirculated. This process continues until such time that all of the powder has been incorporated into the processed fluid and then the valve beneath the funnel is closed. If this valve is not closed a large amount of air can be sucked into the mix creating foam which is undesirable (Kilara & Chandan, 2008).

In the second process, a high shear mixer that functions like a giant Waring blender is used. Here the process fluid is filled to 75% of the volume of the blender. The motor is turned on and under vigorous agitation the dry ingredients are incorporated into the mix. Once all the dry ingredients are incorporated the mixture is discharged into a vat. The hardest ingredients to incorporate are the stabilizers and emulsifiers. If they are not properly handled they form lumps and are not uniformly dispersed in the mix. Excess agitation is undesirable in suspending these ingredients. Generally, mixing stabilizers with dry sugar and corn syrup solids aids in a uniform hydration and suspension of these ingredients (Kilara & Chandan, 2008).

20.2.3.2 Pasteurization

Pasteurization is a heat treatment of food products to destroy pathogenic (disease-causing) microorganisms. According to the US Public Health Service and its Pasteurized Milk Ordinance, pasteurization of an ice cream mix requires that every drop of mix be heated to 68.3°C (155°F) and held at that temperature for 30 min. Alternately, every drop of mix should be heated to 79.4°C (175°F) and held at that temperature for 25 s. Pasteurization can be performed either as a batch operation or as a continuous operation (Anon., 2011).

Batch pasteurization is carried out in a specially designed and approved vat. A batch of mix is placed in the vat and pasteurized by heating the mix to a minimum of 68.3°C (155°F); once that temperature is attained, it is held for 30 min prior to homogenization and cooling. This process is also known as the low-temperature long-time (LTLT) method of pasteurization. Alternatively the HTST method uses 79.4°C (175°F) temperature at 25 s (Anon., 2011).

In the continuous process, a plate heat exchanger is used. This heat exchanger has three sections: (i) regeneration, (ii) heating, and (iii) cooling. In this process the mix is heated to a minimum temperature of 79.9°C (175°F) and held for 25 s; raw cold mix enters the regeneration section where it is heated by the hot pasteurized mix. The warm raw mix is then homogenized and heated by hot water to 79.9°C (175°F). The heated mix flows through a tube called the holding tube for 25 s. Then it has to pass through two controls known as the flow diversion devices. If the mix is cooled to below 79.9°C (175°F) during the holding, the flow diversion devices sense this and send the mix to be re-pasteurized. Once the mix successfully flows past the flow diversion devices, it enters the regeneration section where it gives off some of its heat to the incoming raw mix. Thus the regeneration section is an energy saving device. The pasteurized side of the plate is maintained at a minimum pressure differential of 2 psi (13.79 kPa) so that the raw mix cannot contaminate the pasteurized mix. The partially cooled mix then goes to the cooling section where it is cooled to 4°C (40°F) (Anon., 2011).

20.2.3.3 Homogenization

Homogenization of an ice cream mix results in a smoother eating ice cream. It is a process whereby fat droplets in the mix are reduced to a uniform size. In unhomogenized ice cream mix, the average fat droplet size would be around 2–4 μm. Homogenization breaks down the fat globules to an average size of 1 μm or less. To homogenize the ice cream mix, all the fat must be in the liquid state. Therefore, homogenization is performed on hot mix. In a LTLT (batch) pasteurization system, the homogenization is done after the heating and holding of the mix at a temperature of around 68.3°C (155°F). In the HTST system of pasteurization, homogenization can be performed either after the mix is warmed up in the regeneration section where mix temperature is around 62.7°C (145°F) or after the mix is pasteurized prior to entering the regeneration section when the temperature is 79.9°C (175°F). It is preferable to homogenize prior to pasteurization. The typical pressures for homogenization are 13.79 MPa (2000 psi) first stage and 3.45 MPa (500 psi) second stage. In high-fat mixes, high-acid mixes, chocolate mixes, and mixes with high amounts of egg yolk, homogenization pressures are reduced to 8.27 MPa (1200 psi) first stage and 3.45 MPa (500 psi) second stage (Kilara & Keeney, 1989).

20.2.3.4 Aging

The pasteurized, homogenized mix then is aged in a refrigerated vat. Aging is a process of quiescent storage of the mix with intermittent agitation for a period varying from 3 to 16 hours. During the aging process, the fat crystals that melted during pasteurization recrystallize. The gums or stabilizers also complete their hydration process and the proteins complete their adsorption at the fat/water interface. In the days when gelatin was used as the primary stabilizer in ice cream, aging times of 12–20 hours were recommended. Modern day stabilizers do not use gelatin and require far less time to complete their hydration. A minimum aging time of 2–4 hours is recommended (Kilara & Chandan, 2008).

20.2.3.5 Flavors

Flavor is the most important aesthetic attribute of a food and ice cream is no different. Ice cream differs from other food products in that it has no pre-conditioning flavor like some other foods do. The flavor of ice cream will only become apparent when the product is in the mouth and undergoes melting. One can smell ice cream and not be able to discern the flavor of the frozen product. The flavor is composed of two important attributes, namely taste and

aroma. In the tasting of ice cream, all five senses are used. The sense of sight is used to determine the color of the product, homogeneity of the product and sometimes one may observe ice crystals at the surface. The sense of touch is employed because when ice cream enters the mouth one can sense its temperature that it is cold and smooth. The sense of hearing may also be employed as the ice cream is moved around in the mouth and masticated sound travels along the jawbone to the ear canal and such things as crunchiness of ice crystals can actually be heard. The other two senses, smell and taste are the basis of flavor experience. Perception of aroma is affected by the composition, physical structure, and temperature of the food. Undesirable flavors are called off-flavors. Off-flavors affect the overall flavor qualities of the food. Deteriorative reactions are time dependent and cumulative. Therefore, the length and conditions of storage has a profound influence on the perception of overall flavor. Deteriorative reactions occur in ingredients used in ice cream manufacture. Therefore careful attention should be paid to the quality of ingredients used in ice cream manufacture.

In eating ice cream, whether you lick, bite, or chew, the ice and fat melt. Melting of these two constituents leads to the collapse of the air cell. Upon collapse of the air cell, flavor volatiles are released. Flavor volatiles traverse the palette and enter the olfactory membrane. The brain then recognizes the signal and processes it. An ice cream mix is compounded and processed to obtain a neutral flavored base. This base has the ability to acquire any characterizing flavor added to it. This neutrality of flavor also means that if the mix is mishandled it can easily absorb off-flavors. MSNF sources contribute a slightly salty note but can also contribute to stale, caramelized, old ingredient flavor notes. Off-flavors from whey solids and buttermilk solids should be avoided by using fresh supplies of these ingredients. The most common sweetener used is sugar. Sugar helps augment certain flavors. Corn syrup solids and high-fructose corn syrup solids could contribute a syrupy flavor and may mask the delicate flavor notes of some other ingredients. Mix processed in batch processors is particularly prone to this syrupiness. Stabilizers rarely pose flavor problems but by increasing the viscosity may slow the release of delicate flavors. Emulsifiers rarely pose flavor problems unless they are old. Rotating stocks and inventory control can avoid these problems (Kilara & Chandan, 2008).

Flavors are added in at least three different ways: (i) directly to the mix prior to freezing (e.g., vanilla, chocolate, mint), (ii) immediately after freezing (fruit pieces, nuts, candy, and confectionery pieces), or (iii) after freezing prior to packaging (ripples and variegates). Modern flavoring systems are complicated and may use all three of these modes of flavoring in the same ice cream. The most popular flavors are vanilla, chocolate, fruits, nuts, bakery goods, confectionery items, and ripples or variegates. Nearly 30% of the ice cream manufactured is with vanilla. Single-fold vanilla extract must contain 386.4 g (13.8 oz) of vanilla bean material in 70 proof alcohol. Ice cream made with pure vanilla extract is labeled Category I vanilla ice cream. Ice cream flavored with a mixture of vanilla extract and vanillin (a mixture of natural and artificial with the natural predominating) is labeled as Category II ice cream or vanilla flavored ice cream. A third type called Category III consists of any vanilla that is not Categories I and II. Such a product is labeled as artificially flavored vanilla ice cream (Kilara & Chandan).

Chocolate is another popular flavor. Cocoa beans contain fat and other materials. Once fat is extracted cocoa powder is the residue. Cocoa powders can either contain 10/12% fat or 22/24% fat. Cocoa powders still contain fiber, which can be removed by alkalizing the powder. This process is also known as "Dutch Cocoa." The most common flavoring material is 10/12 cocoa for light, low- and non-fat ice creams whereas 22/24% cocoa is used for regular ice cream (Kilara & Chandan, 2008).

Fruit flavors are popular and can be added as extracts, or as essences often with other natural flavors. Fresh fruit of good quality can also be added and slightly overripe fruits are preferred for this purpose. However, fruits are seasonal horticultural products. The allure of fruit flavored ice cream is to eat it when the fruits are not in season. To accomplish this fruits are sliced and packed with sugar and frozen. Generally one part of sugar is added to three parts of fruit (3 plus 1 pack) or one part of sugar per four parts of fruit (4 plus 1 pack). Sugar is added to protect the fruit during the freezing process. The frozen fruits have to be thawed prior to adding to ice cream. Some fruits such as, strawberries, cherries, pineapple can be heat treated and the flavor improved due to the heat treatment. Stabilized packs need not be refrigerated and some stabilized fruits may have a jam-like flavor rather than that of the fresh fruit (Kilara & Chandan, 2008).

Nuts like pecans, almonds, walnuts, cashews, hazelnuts, peanuts, macadamia, and pistachios are also used to flavor ice cream. Nutmeats must be free of shells, clean, fresh (free of rancidity), and should have low microbial counts. Nuts are generally roasted and salted to keep them fresh. Nuts contain a large amount of unsaturated fatty acids which are susceptible to rancidity. The best results are obtained by using fresh roasted and salted good-quality nuts (Kilara & Chandan, 2008).

Ripples or variegates are a method of flavoring ice cream which incorporates unusual appearance and flavor into ice cream. A good ripple is soft flavorful and distinctive. Sugar

present in the ripple may affect the freezing and storage characteristics of ice cream. Most ripples have stabilizers to impart viscosity. Ripples are introduced into the product by one of two methods: (i) freezer whipping or (ii) pumps other than the freezer. Whipping in the freezer involves double duty for the equipment. The freezer first freezes the ice cream and is then slowed down to incorporate 10–12% of variegating sauce. Air actuated pumps are also used to pump the ripple sauce into the ice cream just prior to packaging. Variable speed controls on such pumps allow different amounts of ripple sauce to be deposited into the product. Ripples have been of such varied flavors as chocolate, marshmallow, peanut butter, butterscotch, caramel, fudge, raspberry, blueberry, or other fruits (Kilara & Chandan, 2008).

Candy and confectionery pieces have become popular flavoring materials in ice cream. Toffees and hard candies are popular flavors. Hard candies have a moisture content of less than 2% and need to be stored properly in order for them to be added without difficulty into ice cream. In the ice cream these pieces should have a clean bite rather than a sticky, tacky one. The candy pieces must be sufficiently large to retain their piece identity in ice cream (Kilara & Chandan, 2008).

Baked pieces like cookies, cookie dough, cakes, pie crusts, etc., are also used as flavorings. Over a period baked goods absorb moisture from the ice cream and become soggy and lose their freshness. Some baked items are fragile and end up as dust in the product. This is not desirable.

20.2.3.6 Freezing

As the refrigeration is turned on and the mix is agitated, it soon reaches its freezing point and nucleation takes place, followed by freezing of some water. Once some of the water is converted to ice, the concentration of the solutes increases and a new freezing point is established. The refrigerant removes some more heat and the new freezing point is reached, nucleation occurs and some more water is frozen. Once again, the concentrations of the solutes increase and yet another freezing point is established and the process is continued until the desired amount of water is frozen. Because this water is frozen rapidly and under agitation small ice crystal nuclei are formed. In the freezing of an ice cream mix, approximately 50% of the water in the mix should be frozen as quickly as possible (matter of minutes). The freezing of half of the water in the mix in a rapid manner results in a large number of small ice crystals. This is desirable in creating a smooth textured ice cream (Kilara & Chandan, 2008).

20.2.3.7 Overrun

In addition to the freezing process agitation helps in the incorporation of air into the ice cream. Incorporation of air leads to a volume expansion of ice cream. The term "overrun" is used to describe the increase in volume of the ice cream. Thus if 3.75 L (1 gallon) of mix is converted to 7.5 L (2 gallons) of ice cream, the volume of the mix is effectively doubled. This is termed 100% overrun. In ice cream operations, a target overrun is chosen for the product and then package weights are calculated. For example, if it is desired to make an 85% overrun ice cream and a gallon of mix weighs 4.14 kg (9.1 lb), the weight of 3.75 L (1 gallon) of 85% overrun ice cream is calculated by the formula: 4.14/1.85 = 2.24 kg or 9.1/1.85 = 4.92 lb. From this calculation the weight of a half gallon of this ice cream can be set at 1.12 kg (2.46 lb), a quart of this ice cream should weigh 0.560 kg (1.23 lb) and a pint should weigh 0.277 kg (0.61 lb).

20.2.3.8 Types of ice cream freezers

Generally, ice cream can be frozen in a batch or a continuous mode. Batch freezers are commonly used by small ice cream shops that make ice cream on the premises. In batch freezers a predetermined amount of mix is charged into the freezing chamber; refrigeration is turned on as is the agitation. Generally, the mix will occupy half of the barrel. The mix is agitated and whipped while being cooled. After some time the mix begins to freeze and when it achieves a certain consistency it begins to incorporate air. Incorporation of air in conjunction with the freezing stiffens the ice cream. At this point the refrigeration should be turned off and agitation continued for some additional period of time. When the desired overrun is achieved, the ice cream is discharged from the barrel with the agitator mechanism still on. Just prior to discharge of the ice cream, fruits and nuts can be added to the barrel, but the preferred method of addition of particulate inclusions is to fold them into the ice cream as it is being discharged from the barrel. Once this process is complete, the next batch of mix can be charged into the freezer barrel and the process repeated. The important variables are the composition of the mix, temperature of the mix, desired overrun, refrigerant temperature, the type and model of the freezer and condition of the scraper blades in the agitation mechanism (dasher). Under ideal conditions a batch of mix should be frozen in 8–10 min. There is some skill needed in operating such a freezer and batch-to-batch variations are routine in such products (Kilara & Chandan, 2008).

Continuous freezers are commonly used in larger ice cream manufacturing plants where more than 1875 L (500 gallons) of ice cream per day may be manufactured. Continuous ice cream freezers have larger capacities, can be operated continuously, ingredients can be added in-line, and packaging can also be automated. Continuous freezers make it possible to produce ice cream of different shapes

through extrusion devices. Novelty extrusions such as sandwiches, pre-filled cones and cups, cakes, etc., are possible through the use of continuous freezers. The ice cream from a continuous freezer is smoother and creamier than a product from a batch freezer. This is because the ice crystals formed in a continuous freezer are smaller and the air cells may also be more uniform. The ice cream exiting a continuous freezer is also generally colder than that coming out of a batch freezer. There are a number of different types of continuous ice cream freezers: some are vertical freezers, especially for smaller scale operations, others are horizontal ice cream freezers. Regardless of whether the freezing cylinder is horizontal or vertical all continuous freezers have a set of blades for scraping the walls of the freezers. In a continuous freezer a mixture of air and mix is introduced at one end and is progressively frozen until ice cream is discharged at the other end. The conveyance of the mixture of air and mix and the discharge of the ice cream may be facilitated by coordinated pumps in some models. Also the newer models of freezers are equipped with microprocessor controls that monitor and control the discharge temperature of the ice cream, the viscosity and the overrun of the ice cream. These microprocessors can work in tandem with other downstream equipment such as ingredient feeders and packaging lines (Kilara & Chandan, 2008).

In continuous freezers the air for the overrun has very little effect in the freezing cylinder because it is compressed. In a freezer operating under 4 atm (59 psi, 407 kPa) cylinder pressure, the air required to give 100% overrun occupies only 15% of the volume of the total mix. The density of the mixture in the freezer is not altered enough by the air to affect the rapid internal heat flow to the cylinder walls. When the semi-frozen ice cream exits the freezer barrel, it expands as the pressure is lowered to atmospheric and when this expansion has been completed maximum overrun is achieved (Kilara & Chandan, 2008).

Continuous freezers enable production of ice cream of high overrun and low drawing temperatures. Air for overrun of up to 130% at draw temperatures of –7.2°C (19°F) can be achieved with cylinder pressures of 3.5–5.5 atm (50–80 psig, 345–552 kPa) depending upon the dasher and blade design and the condition of the blades. For overrun in excess of 130%, cylinder pressures may have to be increased further. When draw temperature is lower than –7°C (19°F) cylinder pressures may have to be increased by 2–3 atm (29.4–44 psi, 203–304 kPa).

The temperature of the mix entering the freezer is very important to freezer performance. If the temperature of the mix is uniform throughout the run, the overrun control and freezing rate are predictable, provided that the refrigerant supply and suction conditions are uniform. Mix temperatures of 0°C (32°F) will optimize freezer performance. However, to achieve such a low temperature of the mix a scraped surface heat exchanger may have to be used. Normal pasteurized mix temperatures are around 3–4°C (~40°F). Newer freezer designs make it possible to extrude ice cream at –18°C (0°F) creating some interesting and desirable texture characteristics (Kilara & Chandan, 2008).

The consistency of ice cream as it is drawn from the freezer is often referred to as "wet" or "dry" or "stiff." Mix that produces a characteristic wet ice cream can be reformulated to produce a dry product. Stiffer drier ice cream is advantageous when manufacturing novelties where the ice cream is manipulated to form different shapes. Flowable wet ice cream is preferred when filling containers of various sizes, because such a product results in a uniform fill with no empty pockets. Stiff, dry ice cream when filled in containers can leave voids that consumers interpret as companies cheating them (Kilara & Chandan, 2008).

The capacity of continuous freezers is difficult to rate since frozen desserts have differing characteristics which in turn affect refrigeration requirements. There is no generally adopted standard among equipment manufacturers for rating freezer throughput. However, if the machines are new or in excellent operating conditions, refrigerants are oil free, the ice cream mix is approximately 10% fat, 15–16% sugar, 37–38% total solids, and the mix enters the freezer at 4°C (40°F), exits at –5°C (23°F) and the refrigerant evaporating temperature is –5°C (23°F) or 2 psig pressure (13.79 kPa), a valid comparison can be made for ice cream throughput at 100% overrun. It is critical to have all conditions to be the same to make valid comparisons between manufacturers of equipment. The rating is a nominal value and it is a given that the ice cream manufacturer will rarely approach these ratings in day to day production (Kilara & Chandan, 2008).

20.2.3.9 Hardening

The aim of freezing ice cream is to convert approximately 50% of the water in the mix to ice. This is done by rapid freezing in the continuous freezer, which also results in small ice crystals. The remainder of the water in the mix is frozen on to these newly created ice crystals as rapidly as possible in an operation called hardening. The time to harden is affected by the package size and geometry, air temperature, air velocity, and turbulence, package surface exposure to cold air and over-wrapping, bundling etc. In order to harden ice cream the package of ice cream is placed in a very cold environment where large volumes of very cold air sweep the surfaces of the packages for a period of time. In such instances freezing of the remaining water on the already existing nuclei takes place from the outside towards the center of the package. As more water

gets converted to ice it acts as an insulator. Therefore, it takes a considerable amount of time for the center of the package to reach –18°C (0°F). It is recommended that the center temperature of a rectangular 1.875 L (half gallon) of ice cream reach –18°C (0°F) in 3 hours or less. In order to achieve this, the air temperature has to be at least –28.9 to –34.4°C (–20 to –30°F). A larger package, such as an 11.25-L (3 gallon) tub, will take a longer period of time to reach a center temperature of –18°C (–0°F). Ideally a core temperature of –18°C (0°F) should be reached in 9–10 hours. Hardening apparatus configurations can be a room, tunnels, spiral tunnels, straight through tunnels, contact plate freezers, and special tunnels used in novelty manufacture. Hardened ice cream is stored at –28°C (–20°F) prior to distribution (Kilara & Chandan, 2008).

20.2.4 Frozen yogurt

Frozen yogurt is labeled according to the fat content of a standard serving size (4 fl oz; 118 mL) used in the ice cream industry. Accordingly, the product containing more than 3 g of fat per 118 mL is labeled as frozen yogurt; the product containing 0.5–3.0 g per 118 mL is low-fat frozen yogurt, and the product with less than 0.5 g of fat is labeled non-fat frozen yogurt.

Some manufacturers may pasteurize the soft frozen yogurt mix, which is a low-acid food, to enhance its shelf-life. Pasteurization also assures safety of the food by destruction of possible contaminating pathogens, including *Listeria* and *Campylobacter*. However, the label of the heat-treated product must display the parenthetical phrase "heat-treated after culturing" on the package panel.

A typical process for making frozen yogurt involves making appropriate mix followed by a freezing process similar to ice cream processing. Like ice cream, frozen yogurt is flavored and extruded from ice cream freezer at –8°C to obtain soft serve frozen yogurt for immediate consumption.

Soft serve frozen yogurt may be garnished with nuts and other food materials to enhance its eating experience. The extruded frozen yogurt may be packed in suitable containers and hardened at –25°C to obtain hard pack frozen yogurt. The ice cream freezer is a scraped surface freezing barrel (heat exchanger). As the liquid mix is pumped through the barrel, removal of the sensible and latent heat leads to formation of frozen mass. The dasher scrapes the inner surface of the barrel while the frozen mass moves toward the exit point. Simultaneously, air cells are formed as a result of whipping action of the dasher and the volume of the mix increases. Eventually, the semi-frozen yogurt mass exits from the barrel as foam with a specific controllable degree of aeration. The overrun or the degree of air incorporated in the foam is around 50%. It implies that the original volume of the mix is increased by 50% in the finished frozen yogurt.

20.2.5 Packaging

For frozen dessert packaging three main factors have to be considered: (i) the package has to protect against temperature fluctuations, photo-oxidation, dehydration and odor transmittance; (ii) it has to take into consideration distribution related factors such as package integrity, thermal shock and cube efficiency; and (iii) municipal solid waste management factors have also to be considered.

Regardless of the container shape and material of construction, ice cream packages are often shrink-wrapped and then sleeved in singly or in pairs prior to entering the hardening systems. The shrink wrap is an indication of tampering but it also provides an additional layer of protection. It is a two-edged sword in the sense that in addition to providing an extra layer of protection, the heat applied to seal may cause heat shock and, more importantly, reduce heat transfer rates during the hardening phase of manufacture. This can result in longer times for hardening ice cream and act as a capacity constraint (Kilara & Chandan, 2008).

20.3 NUTRITIONAL PROFILE OF ICE CREAM

Since ice cream and frozen yogurt are largely composed of milk, the nutritional profile of milk is carried over to these milk products.

20.3.1 Contribution of milk

Dairy products are superior sources of calcium, protein, magnesium, potassium, niacin (B_6), cyanocobalamin (B_{12}), riboflavin (B_2), phosphorus, vitamins A and D, and essential fatty acids. From a public health point of view milk provides three of four nutrients of concern, namely calcium, vitamin D, and potassium. Three cups of fat-free milk (24 fl oz, 720 mL) provide the following percentages of the daily values for key nutrients: calcium 90%, potassium 33%, phosphorus 74%, protein 50%, vitamin A 30%, vitamin D 86%, vitamin B_{12} 61%, riboflavin 79%, and magnesium 20% (Heaney *et al*., 2010).

Various nutritionally advantageous effects of dairy product consumption on bone health, weight management and cardiovascular health have been reported. The US Surgeon General's Report (2004) on bone health stresses the importance of calcium in building strong bones. Populations avoiding dairy have been shown to have lower bone densities and a greater propensity for fractures (Obermayer-Pietsch *et al*., 2004). Consumption of calcium supplements is not the same as obtaining this mineral through a dairy product, because the supplement lacks the other key nutrients supplied by dairy foods. Furthermore, calcium from

Table 20.9. Distribution of amino acids in cows' milk.

Amino acids	Concentration in 2% reduced fat milk (mg per serving of 244 g)	Concentration in milk-solids-not-fat* (mg/g)	Recommended daily allowance for adults† (g/day)
Essential			
Histidine	230	10.31	0.98
Isoleucine	517	22.98	1.3
Leucine	835	37.16	2.9
Lysine	676	30.12	2.7
Methionine‡	213	9.5	1.3
Phenylalanine§	412	18.34	2.3
Threonine	385	17.12	1.4
Tryptophan	120	5.34	0.35
Valine	571	25.39	1.68
Non-essential			
Alanine	294	13.1	—
Arginine	309	13.76	—
Aspartic acid	647	28.79	—
Cystine	78	3.5	—
Glutamic acid	1786	79.48	—
Glycine	181	8.04	—
Proline	826	36.78	—
Serine	463	20.66	—
Tyrosine	412	18.34	—

*Based on 8.67% MSNF for whole milk.
†Values calculated for 70-kg male.
‡Total sulfur-containing amino acids methionine + cysteine.
§Total aromatic amino acids, phenylalanine + tyrosine.
Sources: based on data from Aneja *et al*. (2002) and Miller *et al*. (2007).

non-dairy sources is not absorbed efficiently (Heaney *et al*., 2002; Heaney, 2007).

As far as weight management is concerned, children who consume very little or no dairy are at a higher risk for being obese or overweight (Black *et al*., 2002; Rockell *et al*., 2005). In adults, avoiding or low dairy intake results in increased body fat (Heaney & Rafferty, 2008). High consumption of milk and dairy foods lowers the risk of developing metabolic syndromes (Pereira *et al*., 2002), hypertension (Jorde & Bonaa, 2000; Pereira *et al*., 2002; Elwood *et al*., 2007), and colon and other cancers (Cho *et al*., 2004).

Dietary Approaches to Stop Hypertension (DASH) (Anon., 2006) includes three servings of low-fat milk and dairy products and recommends eating plants high in potassium, magnesium, and protein.

20.3.1.1 Milk proteins

Milk proteins constitute 38% of the solids-not-fat content of milk and 21% of the energy of whole milk. They are recognized as high-quality proteins, contributing approximately 19% of the US food supply of protein.

Milk protein contains all the nine essential amino acids which the human body cannot synthesize. Therefore, the essential amino acids must be furnished by the diet. Table 20.9 shows the recommended daily allowances for adults as well as the amino acids contributed by 2% reduced fat milk to the diet (Miller *et al*., 2007). Both essential and non-essential amino acids are shown.

The quality of a protein is expressed in several ways. Milk protein and its fractions display outstanding nutritional quality as determined by different measurements. Table 20.10 shows the data to support this claim. Both caseins and whey proteins of milk possess physiological and biological properties. The biological properties of milk proteins are summarized in Table 20.11.

20.3.1.2 Milk fat

Milk fat in freshly secreted milk occurs as microscopic globular emulsion of liquid fat in the aqueous phase of

Table 20.10. Comparative nutritional measures of the quality of various food proteins.

Protein	PER	AAS	BV	PD	PDCASS	NPU
Milk protein	3.1	1.27	91	95	1.21	86.45
Casein	2.5	1.24	77	100	1.23	76
Whey protein	3.2	1.16	104	100	1.15	92
Whole egg	3.9	1.21	100	98	1.18	94
Soy protein conc.	1.7	0.96	—	95	0.91	—
Wheat flour	0.6	0.38	61	91	0.42	56
Rice, polished	2.2	0.66	64	—	—	59

PER, protein efficiency ratio: gain in body weight divided by weight of protein consumed by growing rats fed 10% (w/w) of test or reference protein.

AAS, amino acid score: content of the first limiting essential amino acid in test protein compared with the content of that essential amino acid in a reference pattern of essential amino acids.

BV, biological value: proportion of absorbed protein that is retained for body maintenance and/growth.

PD, protein digestibility: proportion of food protein absorbed.

PDCASS, protein digestibility corrected amino acid score: ratio of mg of limiting amino acid in 1 g of test protein and mg of the same amino acid in reference requirement pattern multiplied with true digestibility:

True digestibility: $I(F - f)/I$, where I represents nitrogen intake, F total nitrogen excretion, and f fecal nitrogen excretion on a protein-free diet.

NPU, net protein utilization: proportion of protein intake that is retained, calculated as BV × PD.

Sources: based on data from Schaafsma & Steijns (2000), Southward (2002) and Miller *et al*. (2007).

Table 20.11. Bioactivity of milk proteins.

Protein	Physiological effect
Caseins	Precursors of bioactive peptides
	Calcium and phosphorus carrier
Whey proteins	Confer passive immunity for disease prevention
	Reduce risk of heart disease and lower blood pressure
	Antiviral and anticancer activity
	Control of gut microflora
	Control of cellular glutathione level
β-Lactoglobulin	Binds zinc, calcium and fat-soluble vitamins
	The branched-chain amino acids enhance immune system
α-Lactalbumin	Lactose synthesis in mammary gland
	Anticarcinogenic and immune-enhancing effects
	May be associated with stress reduction: increases serotonin production in brain, improves mood and decreases cortisol level
IgA, IgM, IgG1, IgG2	Antibodies against diarrhea and gastrointestinal tract disturbances
	Support passive immune function
Bovine serum albumin	Antioxidant and antimutagenic
	Binds free fatty acids and pro-oxidant transition metals
Lactoferrin	Bacterial antitoxin binding, antibacterial, antiviral, immune modulating, anti-inflammatory
	Antithrombotic activity, anticarcinogenic, antioxidant, iron absorption
Lactoperoxidase	Antimicrobial, antioxidant, immune-enhancing properties
Lysozyme	Antimicrobial, synergistic with immunoglobulins and lactoferrin

Sources: based on data from Harper (2000), Hoolihan (2004) and Chandan (2007, 2011).

milk plasma. The composition of milk fat is given in Table 20.12. The milk fat of cows consists chiefly of triglycerides of fatty acids, which make up 95–96% of milk fat. The remaining milk fat is composed of diglycerides, monoglycerides, free fatty acids, phospholipids, and cholesterol. The functional properties of milk fat are attributed to its fatty acid composition. More than 400 distinct fatty acids have been detected in milk. Typical milk fat consists of 62% saturated, 29% monounsaturated, and 4% polyunsaturated fatty acids. In view of the atherogenic activity of saturated fat like milk fat, medical authorities have restricted its intake. The industry has responded with the introduction of low-fat and non-fat dairy foods. It is interesting to note that milk fat contains 7–8% short-chain fatty acids (C4–C8), which is a unique characteristic of milk fat. Previously it was thought that high consumption of dairy foods might increase the risk for cardiovascular disease. The current evidence, however, points to an opposite effect, i.e., high consumption of dairy foods decreases the risk for cardiovascular disease (Elwood *et al*., 2007; German *et al*., 2009; Goldbohm *et al*., 2011). A summary of some beneficial effects of milk fat is shown in Table 20.13.

Table 20.12. Constituents of bovine milk lipids.

Lipid fraction	g/L whole milk	Milk fat weight (%)
Triacylglycerols/triglycerides	30.7	95.80
Diacylglycerols/diglycerides	0.72	2.30
Monoacylglycerols/ monoglycerides	0.03	0.08
Free fatty acids	0.09	0.28
Phospholipids	0.36	1.11
Cholesterol	0.15	0.46
Cholesterol esters	0.006	0.02
Total	32.056	100.05

Source: reproduced from Chandan (2011), with permission of John Wiley & Sons.

20.3.1.3 Lactose

The major carbohydrate in milk, lactose monohydrate, ranges from 4.8 to 5.2%. Lactose stimulates the absorption of calcium and magnesium. It has relatively low glycemic index of 46 compared with 100 for glucose and 60 for sucrose. This makes lactose in skim milk suitable for diabetics and in weight control diets. It is less cariogenic than other sugars. Lactose malabsorption is prevalent in certain sections of human populations. However, lactose-free milk is commercially available to enjoy the nutritional advantages of milk-associated nutrients, namely milk proteins,

Table 20.13. A summary of health effects of certain milk fat constituents.

Constituent	Physiological effect
Butyric acid	Reduces colon cancer risk
Essential fatty acids (arachidonic acids, linoleic acid (omega-3), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)	Anti-inflammatory Beneficial in preventing heart disease Improve mental function and vision Reduce cancers of prostate, colon and breast
Monounsaturated fatty acid (oleic acid)	Cardioprotective, lower LDL cholesterol
CLA (conjugated linoleic acid)	Modulate immune function Reduce risk of cancer (stomach, colon, breast and prostate) Assist in weight reduction Increase bone density Reduce chronic inflammation Normalize blood glucose level by increasing insulin sensitivity
Phospholipids	Protect gastric mucosa, extend protection from pathogenic organisms
Sphingolipids	May reduce risk of colon cancer Reduce serum cholesterol, elevate HDL (the protective cholesterol) Protect against bacterial infections and toxins
Stearic acid	May modulate blood lipids to reduce risk of cardiovascular and heart disease
Triglycerides	May enhance long-chain fatty acid and calcium absorption

Sources: based on data from Hoolihan (2004), Chandan & Shah (2007) and Chandan (2011).

Table 20.14. Major and minor minerals of whole cows' milk.

	Mean (mg/100 mL)	Range (mg/100 mL)
Major minerals		
Calcium (total)	121	114–130
Calcium (ionic)	8	6–16
Citrate	181	171–198
Chloride	100	90–110
Magnesium	12	9–14
Phosphorus, inorganic	65	53–72
Potassium	144	116–176
Sodium	58	35–90
Trace elements	*μg 100 g milk*	*μg 100 g milk*
Boron	27	—
Chromium	1	0.8–1.3
Cobalt	0.1	0.05–0.13
Copper	20	10–60
Fluoride	12	3–22
Iodine	26	—
Iron	45	30–60
Manganese	3	2–5
Molybdenum	7	2–12
Nickel	2.5	0–5
Selenium	12	5–67
Silicon	260	75–700
Zinc	390	200–600

Sources: based on data from Fox (2002), Chandan & Shah (2007) and Chandan (2011).

minerals, and vitamins. Furthermore, cultured dairy foods, especially yogurt, are well tolerated by lactose malabsorbers, because lactose is converted to lactic acid (Chandan & Kilara, 2008).

20.3.1.4 Minerals

Milk is an excellent source of minerals. The mineral content of milk is given in Table 20.14. Milk and dairy products are excellent sources of bioavailable calcium. Milk supplies assimilable calcium and phosphorus in an optimum ratio. The major source of dietary calcium is dairy products, supplying as much as 75% of the dietary intake in the developed nations. The bioavailability of calcium is further enhanced by the presence of vitamin D, lactose, and phosphoprotein (casein). The primary function of calcium is to provide strength and structural properties to bone and teeth. A lack of adequate calcium intake particularly during the growth phase leads to osteoporosis or brittle bones in later life. It is also important in teeth development (Chandan & Kilara, 2008).

Calcium is involved in muscle contraction (including heart beat), blood coagulation, enzyme reactions, stimulation of hormonal secretions, and neural cell signaling. It is important in blood pressure control and is a factor in the prevention of colon cancer. Phosphorus is also critical in bone mass formation and takes part in various metabolic processes in the body. It is a crucial component of the genetic material DNA and RNA (Chandan & Kilara, 2008).

Iron is essential in the formation of hemoglobin and in cytochrome activity. A deficiency causes anemia. Iron is further involved in brain function, in immunocompetence, and in the synthesis of lipids. Magnesium is also a part of bone mass. It is involved in many metabolic pathways. Zinc is a component of several metabolic enzymes and DNA. It is involved in immune system functioning. Iodine is necessary for the formation of thyroid hormone that regulates growth and metabolism. Copper is important in energy metabolism, as an antioxidant, and is involved in collagen synthesis and iron utilization. Manganese is a cofactor of many metabolic enzymes. Chloride is an oxidizing agent and constitutes a vital ingredient of stomach acid. Potassium is a major electrolyte in blood and tissues and helps in blood pressure regulation in conjunction with sodium. Sodium is further involved in nerve conduction, active transport, and bone formation (Chandan & Kilara, 2008).

20.3.1.5 Vitamins and some other minor constituents

To promote health and well-being, a balance of minerals and vitamins is required. They have to be supplied by food and supplements because they are not manufactured by the body. Milk contains both fat-soluble and water-soluble vitamins. The concentration of fat-soluble vitamins A, D, E, and K, and water-soluble vitamins B and C, and minor constituents of milk are given in Table 20.15 (Chandan & Kilara, 2008).

Natural vitamin A activity in milk is due to retinol and the pigment β-carotene. Their level as well as those of vitamin D and E varies in milk according to the season and feed profile. The richest source of vitamin D in the human diet is vitamin D fortified milk. Exposure to sunshine helps to activate this vitamin. Vitamin D assists in calcium absorption. It helps to form and maintain strong bones. It is also recognized for its role in the prevention of bone disease rickets. More recent research has shown that vitamin D reduces the risk of several types of cancer, and improves immune function. It also gives protection against multiple sclerosis and helps in reducing falls in frail elderly people. Vitamin E is an antioxidant. Vitamin K is present in milk but its dietary nutritional role is probably minor (Chandan & Kilara, 2008).

Milk is an important source of dietary B vitamins. They are stable to various heating and processing conditions milk is normally subjected to. Vitamin B_1, thiamin, is a cofactor in carbohydrate metabolism. Vitamin B_2 is involved in the oxidation reactions of glucose, fatty acids, amino acids, and purines. Niacin facilitates utilization of carbohydrates, fat synthesis, and tissue respiration. Pantothenic acid participates in fatty acid metabolism. Vitamin B_6 is critical in protein metabolism. Folic acid acts as a growth factor and is involved in DNA synthesis. Vitamin B_{12} is required for growth, blood formation, and nerve tissue functioning. Biotin has a role in metabolism of carbohydrates, lipids, nucleic acid, and proteins. Ascorbic acid (vitamin C) is necessary for collagen formation, healing of wounds, and absorption of non-heme iron. It provides resistance to infections. However, vitamin C content of milk is relatively low (Chandan & Kilara, 2008).

Table 20.15. Vitamin concentration of cows' milk.

Vitamin	Concentration in 100 mL of whole milk
Fat-soluble vitamins	
A	40 µg RE
D	4 IU
E	100 µg
K	5 µg
Water-soluble vitamins	
B_1	45 µg
B_2	175 µg
Niacin	90 µg
B_6	50 µg
Pantothenic acid	350 µg
Biotin	3.5 µg
Folic acid	5.5 µg
B_{12}	0.45 µg
C	2 mg

Source: reproduced from Chandan (2011), with permission of John Wiley & Sons.

Traditionally, the nutritional role of milk has been linked to the supply of essential and non-essential nutrients to optimal human growth, development, and sustenance. Currently, more emphasis is being placed on prevention of chronic diseases by dietary and lifestyle changes. In this regard, the role of specific dairy components in providing health benefits encompassing reduction in risks of developing chronic conditions has been emphasized. The disorders of interest are weight management, body fat loss and obesity, bone health and osteoporosis prevention, blood pressure reduction, type 2 diabetes relationship, and combating certain cancers (Chandan & Kilara, 2008).

20.3.2 Nutrient profile of ice cream and frozen desserts

As explained in the previous section, milk contributes calcium, potassium, phosphorus, protein, vitamins A, D, B_{12}, riboflavin, and magnesium to ice cream. In order to compare the levels of these key nutrients in milk and ice cream, data from the USDA Nutrition Laboratory database were reviewed (USDA, 2010). The data are shown in Table 20.16. The values in the table represent nutrients

Table 20.16. Comparison of key nutrients in 100-g portions of cows'milk and ice cream.

	Milk		Ice cream			
Nutrients	**Skim**	**Whole**	**Regular**	**Light**	**Fat free**	**Light soft serve**
Energy (kJ)	143	256	868	755	578	527
Energy (kcal)	34	61	208	180	138	126
Protein (g)	3.37	3.15	3.5	4.78	4.48	4.9
Calcium (mg)	122	113	128	161	149	157
Phosphorus (mg)	101	84	105	103	150	121
Magnesium (mg)	11	10	14	14	21	14
Potassium (mg)	156	132	199	208	302	221
Vitamin A (IU)	204	162	421	448	204	103
Vitamin D (IU)	47	2	8	4	47	0
Riboflavin (mg)	0.182	0.169	0.240	0.255	0.182	0.198
B_{12} (µg)	0.50	0.45	0.39	0.47	0.50	0.50
Fat content (g)	0.08	3.25	11.0	5.0	0	2.5

Source: based on data from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

Table 20.17. Key nutrients in ice cream and frozen desserts expressed as a percentage of the reference values based on a 2000 calorie intake for adults and children over 4 years old.*

Nutrient	Reference value	% Reference value in 100 g ice cream			
		Regular ice cream	Light ice cream	Fat free	Light soft serve
Calcium (mg)	1000	12.8	16.1	14.9	15.7
Magnesium (mg)	400	3.5	3.5	5.25	3.5
Protein (g)	50	7	9.6	9	9.8
Vitamin A (IU)	5000	8.4	9.0	4.1	2.1
Vitamin D (IU)	400	2	1	11.75	0
Vitamin B_{12}(μg)	6	6.5	7.8	8.3	8.3
Riboflavin (mg)	1.7	14.1	15	10.7	11.6

*Reference values for potassium and phosphorus have not been established and are not included in this table. Values calculated from nutrient tables based on data from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

present in 100-g portions of the products. Atypical serving of milk is one cup (8 fl oz or 240 mL) and a serving of ice cream and related frozen desserts is 0.5 cup (4 fl oz or 120 mL). Since serving sizes are volumetric, a 100-g portion represents approximately 40.4% of a serving of milk and 80.75% of one serving of ice cream. Ice cream and frozen dairy desserts contain air (overrun) and if the normal overrun is assumed to be 100% it means that in a serving of ice cream approximately 50% is air by volume. Air does not contribute nutrients or calories. A 100-g portion of ice cream at 100% overrun translates to 200 mL or 6.7 floz cup. It is recognized that the density of ice cream is not a constant and is dependent upon the composition and the overrun.

On an equivalent weight basis, ice cream contains more energy, protein, calcium, magnesium, phosphorus, potassium, and riboflavin than skim and whole milk. It can be considered a nutrient-dense food and equally a calorie-dense food. Addition of fruits, nuts, variegated, candy, and baked pieces to ice cream will increase the calorie density. Lowering the overrun will have a similar effect. Both nuts and fruit are regarded as healthy ingredients. Nuts contribute healthy fats, minerals, and protein. Fruits are known to be healthy sources of antioxidants, fiber, minerals, and vitamins. Their addition to ice cream supplements nutrition and enhances the health attributes of ice cream.

Another way of looking at nutrient contribution is to determine the contribution of a product as a percentage of the reference value for that nutrient (Table 20.17). Nutrient content claims allow for "good source" if 10–19% of the daily value is provided in a serving of the product and "excellent source" if the serving provides 20% or greater of a nutrient in a serving. Based on these criteria 100-g portions of ice cream products can make "good source" claims for calcium and riboflavin.

Table 20.18. Energy and nutrient content in one serving (½ cup) of various types of ice creams.

Nutrient	Fat free	Light	Regular	Super premium
Energy (kcal)	90	137	140	270
Protein (g)	3	4	2	4
Lipids (g)	0	4	7	18
Carbohydrate (g)	19	22	16	24
Fiber (g)	5	0	0.5	0
Sugar total (g)	6	17	14	22
Calcium (mg)	100	120	85	125
Iron (mg)	0	0	0	0
Sodium (mg)	75	60	55	65
Vitamin C (mg)	0	1	0	0
Vitamin A (IU)	315	340	280	700
Cholesterol (mg)	0	21	30	100
Saturated fat (g)	0	2.2	4	11
Unsaturated fat (g)	0	1	2	5

Source: from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

If the nutrients that are often considered as negative for health are compared for various frozen desserts, it becomes clear that there is no need for concern (Table 20.18). It is also instructive to compare ice cream with selected desserts (Table 20.19).

Various attempts at improving the nutritional properties of ice cream have been reported in the literature. These include addition of omega-3 fatty acids, fiber, prebiotics,

Table 20.19. Nutrient composition of some commonly consumed desserts.

Dessert	Vanilla pudding (½ cup)	White cake (74 g)	Carrot cake (85 g)	Cheesecake (80 g)	Apple pie (85 g)	Pumpkin pie (85 g)
Energy (kcal)	160	265	355	260	250	210
Protein (g)	4	4	4	7	2	3
Lipid (g)	4	9	8	28	10	8
Carbohydrate (g)	27	42	67	32	38	30
Fiber (g)	0	0	0	1	1	2
Sugars (g)	24	26	ND	27	19	16
Calcium (mg)	140	96	146	41	12	54
Iron (mg)	0	1	153	1	1	1
Sodium (mg)	220	240	480	350	170	203
Vitamin C (mg)	0	0	1	0	5	0
Vitamin A (IU)	200	40	1640	440	70	2920
Cholesterol (mg)	13	1	0	70	0	0
Total saturated fat (g)	2	2	1	12	2	2
Total unsaturated fat (g)	1	6	7	13	7	7

Source: reproduced from USDA National Nutrient Database for Standard Reference, Release 23 (2010).

Table 20.20. Proximate composition of milk from various mammalian species compared with bovine milk.

Species	Water (%)	Protein (%)	Fat (%)	Lactose (%)	Ash (%)
Cow	86.6	3.4	4.6	4.9	0.5
Water buffalo	84.2	3.2	6.6	5.2	0.8
Goat	86.5	3.5	4.5	4.7	0.8
Horse	89.1	2.7	1.6	6.1	0.5
Sheep	79.4	6.7	8.6	4.3	1.0
Camel	86.5	4.0	3.1	5.6	0.6
Donkey	90.0	1.7	1.3	6.5	0.5
Yak	82.7	5.8	6.5	4.6	0.8

Source: reproduced from Pritchard & Kailasapathy (2011), with permission of John Wiley & Sons.

probiotics, protein, enzymes, and fats with higher proportions of unsaturated fatty acids. Technically all such enhancements are successful; however, commercial success is hard to achieve because ice cream and frozen dairy desserts are considered indulgent products. By the very thought of indulgence, nutritional enhancements are counterintuitive to consumers. In our combined professional experience of approximately 100 years, "Don't mess with my ice cream" is an often echoed sentiment by consumers. With the introduction of the Nutritional Labeling and Education Act in 1992, categories of low- and not-fat ice cream were introduced to the US market. Within a period of about 3 years, non-fat ice cream is no longer being manufactured in the USA and the consumption of low-fat ice cream has declined. The reason suggested for this change in consumption pattern is the lack of richness of these products.

20.3.3 Frozen dairy products from milk of species other than cow

Cows' milk is the predominant milk used for human nutrition in the world. However, in certain regions of the world milk of other mammals is utilized. Table 20.20 illustrates the comparative composition of milk of various species. Although they differ in chemical composition, milk and various products derived from species other than cow do contribute vital nutrients in the regions specializing in

producing such milks. For instance, in south Asian countries, market milk and the dairy products consumed contain a large proportion of water buffalo milk (Aneja *et al*., 2002). The local dietary patterns adapt to the flavor and texture associated with such milks.

Information on frozen desserts made from milk of species other than cow and buffalo milk is limited. A frozen dairy product called Kulfi has been described by Aneja *et al*. (2002). Kulfi is obtained from cow or buffalo milk or a combination thereof. Dairy ingredients include milk, cream, partially desiccated milk (concentrated by heat), sugars, fruit, nuts, chocolate, flavors, and colors. The product contains not less than 10% milk fat, 3.5% protein, and 36% total solids. When fruits or nuts are used, the product contains a minimum of 8% milk fat. Starch may be used at a maximum of 5%. Kulfi differs from ice cream in that it contains practically no overrun/air. Equal parts of cow and buffalo milk make good Kulfi. Typical Kulfi formulation contains 9% milk fat, 17% MSNF, 1–2% nuts (pistachios, almonds) and 13% sucrose. Saffron is also used for flavor and color. In the traditional process, a buffalo milk–cow milk mixture is concentrated in large open pans over a fire until the volume is reduced to 50–60% of the original volume. The concentrated milk is cooled to room temperature and sugar is added while stirring the mixture. After mixing slivers of nuts and saffron, the mix is filled into aluminum/plastic cones and lids are applied to seal. The cones are immersed in a salt–ice mixture in an earthenware vessel. Vigorous agitation assists in heat transfer and in expediting the freezing process. Agitation of cones whips up a small amount of air and Kulfi develops a minor overrun. Industrial processes involve ice cream freezers and novelty product manufacture.

Information on industrial production of ice cream or frozen dairy desserts from milk of dairy species other than cow and buffalo is almost non-existent except for goat milk frozen desserts (Loewenstein *et al*., 1980). It may be postulated that ice cream products might be possible using milk of all species, with minor modifications of solids and fat levels to get an acceptable product. It should be expected that development of infrastructure (refrigeration, freezing technology, and frozen channel for marketing) would soon lead to frozen desserts based on milk of species other than the cow in many developing countries.

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21
Nutritional Formulae for Infants and Young Children

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21.1 INTRODUCTION

Human milk is widely accepted as the ideal 'gold standard' food for infants. In addition to providing an optimum balance of nutrients, breastfeeding promotes a nurturing contact between infant and mother. Furthermore, human milk contains anti-infective components, which help the infant defend against infections. For these reasons, paediatricians worldwide have recommended that breastfeeding be practised whenever possible. In circumstances where a mother is unable to or decides not to breastfeed, infant formula is a safe and suitable alternative. Infant formula is specially formulated with a composition that is largely based on that of human milk. It often serves as the sole source of nutrition during infancy. Thus, the composition of infant formula must be designed to provide all the essential nutrients required for normal healthy development. The chemical composition of human milk has served as a guide for infant formula composition. As knowledge on the composition of human milk advances, infant formula manufacturers respond by designing products ever closer to human milk. Compounds not found in human milk are often added to infant formulae to achieve physiological outcomes in formula-fed infants similar to those in infants fed human milk. Specially designed formulae are available for infants born preterm or for infants with special nutritional requirements, including those with metabolic or digestive disorders. Beyond the early months of infancy, follow-on formulae and growing-up milks are available for older infants and young children as complementary feeds to an increasingly diversified diet.

21.2 HISTORY OF INFANT FORMULA

Owing to its plentiful supply and relatively inexpensive cost, the majority of infant formulae are based on bovine milk. Schuman (2003) published a concise history of infant formula in which the pioneering work of scientists is discussed. In 1838, Johann Franz Simon highlighted the higher protein content and lower carbohydrate content in bovine milk than in human milk. A few decades later in 1860, another German chemist, Justus von Leibig, developed the first commercial baby food; this product was composed of wheat flour, bovine milk, malt flour and potassium bicarbonate, and was designed for addition to heated bovine milk. In the early twentieth century, physicians had reservations about the nutritional adequacy of such products, and advocated against the use of proprietary formulations. However, by the early 1920s after recognising the complexities involved, physicians began to recommend commercial formulae. Many of the major producers of infant formula can refer to important formula developments at this time, such as modifications to the carbohydrate content of bovine milk, formulae containing vegetable oils and formulae with modified mineral contents. By the 1940s and 1950s, commercially available infant formulae were becoming widely available and were generally accepted by physicians. In the 1960s and 1970s, the use of formulae to feed newborn infants in hospitals became more

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commonplace and regulations and guidelines were established to govern the manufacture, composition and labelling of these nutritional products. Ongoing research into the composition of human milk and biological role of its components will drive the continuous evolution of formulae for infants and young children for the foreseeable future.

21.3 CLASSIFICATION AND REGULATION OF FORMULAE FOR INFANTS AND YOUNG CHILDREN

Regulations governing the manufacture, composition and labelling of infant nutritional products are continuously evolving. Both national and international regulations and guidelines have been designed to ensure an adequate nutritional intake for infants and young children fed commercial formulae. A summary of the nutrient limits for first-age infant formulae for three of the major regulatory bodies, the European Commission, the Codex Alimentarius Commission and the US Food and Drug Administration, is given in Table 21.1.

A first-age infant formula is intended for consumption by infants from birth up to about 6 months of age. Thereafter, when an infant has been introduced to some solid foods, a 'follow-on' formula may be provided as a complementary food source. In situations where infants do not thrive on standard first-age formulae, specialised formulae are available which are categorised under the term 'foods for special medical purposes'. Examples of the latter, many of which will be discussed in more detail in following sections, include formulae for premature and low-birthweight infants, lactose-free formulae, non-milk protein formulae, extensively hydrolysed-protein formulae, anti-regurgitation formulae, high caloric or nutrient-dense formulae and low-phenylalanine formulae (O'Callaghan *et al*., 2011). A generally accepted classification of infant nutritional products is outlined in Table 21.2.

21.4 SAFETY AND QUALITY

Infant formulae are often used as the sole source of nutrition for newborn infants. Not all organs and systems are fully mature in an infant at birth, and as infants develop they may become susceptible to nutritional inadequacy and illness. Manufacturers of infant formulae implement and adhere to rigorous safety and quality protocols to guarantee the product supplied for its sensitive customer is of the highest possible standard. These protocols meet, and in many cases exceed, standards set by the Codex Alimentarius Commission, and they must also comply with all regulations in the countries where the product is manufactured and sold. Additionally, all components from ingredients to packaging materials are extensively tested by suppliers, and tested on receipt at the manufacturing site prior to use by the infant formula manufacturer, to ensure consistency with the highest quality and safety standards. The final product undergoes rigorous testing to ensure that the physical and chemical composition and the microbiological status meet stringent quality criteria.

21.5 PRODUCT RANGE AND FORMULATION

21.5.1 General formulation principles

The initial step in formulation involves setting the nutrient content of the formula to comply with the appropriate regulations and guidelines. The declared nutrient content is a list of nutrients and associated concentrations. Suitable ingredients are selected to provide the desired macronutrient (fat, carbohydrate, protein) content. These ingredients will also contribute certain micronutrients such as minerals and vitamins which are accounted for in the formulation. In some instances, the macro-ingredients actually provide sufficient levels of micronutrients to meet the declared nutrient contents.

However, in most instances, the levels of micronutrients in dairy ingredients are insufficient to meet the declared content and fortification is required. Many labile nutrients such as certain water-soluble vitamins are susceptible to losses during manufacture and storage, so it is necessary to add greater quantities than the declared level to account for such losses. Fortification levels of micronutrients are determined during the development stage, where compositional data are generated to demonstrate that each nutrient is present within the defined limits throughout the proposed shelf-life of the formula. As the shelf-life of infant formulae is typically 1–2 years for liquid sterilised formulae and 2–3 years for powder formulae, an accelerated shelf-life testing programme is typically set up to acquire the nutrient stability data in a shorter time frame. In such programmes, the formula is typically stored at an elevated temperature (typically 37–40°C) for a short defined period to accelerate nutrient decay reactions. The accelerated shelf-life testing programme is based on the concept that the nutrient levels in the formula at the end of this short storage period correspondent with the real-time shelf-life conditions. After reviewing the nutrient stability data, appropriate fortification levels are chosen to compensate for the losses of the labile nutrients.

21.5.2 Milk protein-based first-age infant formulae

In the manufacture of the majority of first-age infant formulae, bovine milk is used as the base ingredient. Although bovine and human milks have similar calorie levels (65–70 kcal/dL) (Fomon, 1993), their macronutrient

Table 21.1 Regulatory limits for first-age infant formulae.

Nutrient	Unit (per 100 kcal)	Codex Alimentarius*		European Union†		USA IFA‡	
		Min.	Max.	Min.	Max.	Min.	Max.
Protein	g	1.8	3.0	1.8	3.0	1.8	4.5
Fat	g	3.3	6.0	4.4	6.0	3.3	6.0
Linoleic acid	mg	300	—	300	1200	300	—
Linolenic acid	mg	50	—	50	—	—	—
Carbohydrate	g	9	14	9	14	—	—
Vitamin A	IU	200	600	200	600	250	750
Vitamin D	IU	40	100	40	100	40	100
Vitamin E	IU	0.5	—	0.5	5.0	0.7	—
Vitamin K	µg	4.0	—	4.0	25	4.0	—
Vitamin B_1	µg	60	—	60	300	40	—
Vitamin B_2	µg	80	—	80	400	60	—
Vitamin B_6	µg	35	—	35	175	35	—
Vitamin B_{12}	µg	0.10	—	0.1	0.5	0.15	—
Niacin	µg	300	—	300	1500	250	—
Folic acid	µg	10	—	10	50	4.0	—
Pantothenic acid	µg	400	—	400	2000	300	—
Biotin	µg	1.5	—	1.5	7.5	1.5	—
Vitamin C	mg	10	—	10	30	8.0	—
Choline	mg	7.0	—	7	50	7.0	—
Inositol	mg	4	—	4	40	4	—
Calcium	mg	50	—	50	140	60	—
Phosphorus	mg	25	—	25	90	30	—
Magnesium	mg	5.0	—	5.0	15	6.0	—
Iron	mg	0.45	—	0.3	1.3	0.15	3
Zinc	mg	0.5	—	0.5	1.5	0.5	—
Manganese	µg	1	—	1	100	5	—
Copper	µg	35	—	35	100	60	—
Iodine	µg	10	—	10	50	5.0	75
Sodium	mg	20	60	20	60	20	60
Potassium	mg	60	180	60	160	80	200
Chloride	mg	50	160	50	160	55	150
Selenium	µg	1	—	1	9	—	—
Nucleotides	mg	—	—	—	5	—	—
Taurine	mg	—	12	—	12	—	—
L-Carnitine	mg	—	1.2	—	1.2	—	—

* Based on data from Codex Alimentarius Commission Standard for infant formula: Standard 72-1981 including amendments and revisions.

† Reproduced from Commission of the European Communities Directive 2006/141/EC on infant formulae and follow-on formulae. © European Union, http://eur-lex.europa.eu/

‡ Based on data from Infant Formula Act of 2003, Code of Federal Regulations, Title 21: Food and Drugs.

profiles are quite different (Table 21.3). The composition of human milk has been reviewed extensively (Jenness, 1979; Ribadeau-Dumas, 1983; Poskitt, 1994; Picciano, 2001; Lawrence & Lawrence, 2011a). Human milk contains less ash and protein and more lactose than bovine milk. Furthermore, there are significant qualitative differences in the composition of the fat, protein and ash fractions of both milks. In the manufacture of infant formulae

Table 21.2 General classification of formulae for infants and young children.

Product	General description	General properties	Intended age bracket	Applications/comments
First-age infant formulae	1. Whey dominant (adapted)	Whey–casein ratio, 60 : 40	From birth to 6 months	Nutritionally complete breast milk substitute for healthy infants
	2. Casein (curd) dominant	Casein–whey ratio, 80 : 20	From birth to 6 months	
	3. Hypoantigenic formulae (HA)	Hydrolysed proteins, 100-fold reduction in allergenicity	From birth to 6 months	Nutritionally complete breast milk substitute for prevention of milk protein allergy
	4. Easy digest formulae	Partially hydrolysed proteins, reduced lactose	From birth to 6 months	Nutritionally complete breast milk substitute for healthy term infants with general formula intolerance issues
	5. Soy-based	Milk protein-free and lactose-free	From birth to 6 months	Nutritionally complete, for treatment of lactose or milk protein intolerance
Follow-on formulae	1. Casein-dominant formulae	Casein–whey ratio, 80 : 20; high in protein, Ca, Fe, and vitamins	From 6 months to 1 year	To be used as substitute for cows' milk. Not necessarily nutritionally complete
	2. Soy-based formulae	Milk protein-free and lactose-free	From 6 months to 1 year	For the treatment of lactose or milk protein intolerance. Used as substitute for cows' milk
Low-birthweight (LBW) formulae	Whey-dominant (adapted)	Whey–casein ratio, 60 : 40; high in protein, Ca, P	From birth as required until progression to PDF or first-age formulae	Nutritionally complete breast milk substitute for LBW or premature infants
Post-discharge formulae (PDF)	Whey-dominant (adapted)	Whey–casein ratio, 60 : 40; high in protein, Ca, P	From hospital discharge of LBW infants as required until progression to first-age formulae	Nutritionally complete breast milk substitute for LBW or premature infants
Foods for special medical purposes	1. Lactose-free (LF)	Whey- or casein-dominant, lactose <0.2 g/100 g powder	From birth to 6 months	Nutritionally complete for treatment of lactose intolerance
	2. Anti-regurgitation (AR) formulae	Casein-dominant, contains starch or other thickeners	From birth to 6 months	Nutritionally complete breast milk substitute for infants prone to gastro-oesophageal reflux
	3. High-caloric or nutrient-dense formulae	Generally whey dominant	From birth to 12 months	Nutritionally complete breast milk substitute for infants small for gestational age, in preoperative or postoperative care
	4. Extensively hydrolysed protein formula	Casein/whey protein hydrolysates	From birth to 12 months	Nutritionally complete breast milk substitute for the management of atopic cows' milk allergy

(*Continued*)

Table 21.2 (*Continued*)

Product	General description	General properties	Intended age bracket	Applications/comments
	5. Low phenylalanine formulae	Casein hydrolysates, <0.08% Phe	From birth to 12 months	Nutritionally complete breast milk substitute for the treatment of phenylketonuria
	6. Elemental diets	Free amino acids	From birth as required	Nutritionally complete breastmilk substitute for the treatment of IgE-mediated cows' milk allergy or atopic infants

Source: based on data from O'Callaghan *et al*. (2011).

bovine milk is modified, or reformulated, to reflect the energy content and nutrient profile of human milk. Diluting the protein content, replacing the milk fat with vegetable oils and altering the mineral and vitamin profile of bovine milk are important features of this reformulation.

21.5.2.1 Energy

During the first few days after birth, a normal healthy infant requires a high energy intake to maintain rapid growth and development. Thus, the energy content of infant formulae is regulated; most first-age infant formulae are formulated with a caloric density similar to that of human milk (65–70 kcal/dL) (Fomon, 1993). In practice, this may be achieved by selecting specific levels of protein and fat and making up the balance of energy with carbohydrate.

21.5.2.2 Protein

Protein provides the infant with amino acids, which are required for the infant's normal growth and development. The essential amino acids that the diet must provide are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine (Fomon, 1993). Cysteine and tyrosine may also be essential for preterm infants. Carnitine, taurine, glutamine and glycine are conditionally essential, whereas other amino acids (alanine, arginine, aspartic acid, asparagine, glutamic acid, proline and serine) are non-essential.

The minimum protein level permitted in infant formulae, regulated by the major regulatory bodies, is 1.8 g/100 kcal (Table 21.1). Further guidelines describe the nutritional quality of the protein. In the EU Directive for infant formulae, it is specified that 'for an equal energy value the formula must contain an available quantity of each essential and semi-essential amino acid at least equal to that contained in the reference protein (breast milk)' (Table 21.4).

Table 21.3 Composition (g/L) of human and bovine milk.

Component	Bovine (g/L)	Human (g/L)
Water	873	878
Fat	37	38
Ash	7	2
Protein	34	10
Casein	28	4
β-Casein	11.2	2.6
κ-Casein	2.8	1.2
Whey protein	6	6
β-Lactoglobulin	3.2	Absent
α-Lactalbumin	1.2	2–3
Serum albumin	0.4	0.9
Lactoferrin	0.01	2–4
Lysozyme	0.0001–0.00035	0.04–0.09
Lactoperoxidase	0.03	Trace
Immunoglobulins	0.8	1
Carbohydrate	48	70
Lactose	40–50	55–70
Lacto-*N*-tetraose	—	0.5–1.5
Lacto-*N*-fucopentaose I	—	1.2–1.7
Lacto-*N*-fucopentaose II	—	0.3–1.0
Lacto-*N*-fucopentaose III	—	0.01–0.2
Lacto-*N*-difucohexaose	—	0.3–0.5
6-Sialyl-lactose	Trace	0.1–0.3
3-Sialyl-lactose	Trace	0.03–0.2
Sialyl-lacto-*N*-tetraose a	—	0.1–0.6
NeuAc2-lacto-*N*-tetraose	—	0.2–0.6

Sources: based on data from Sanchez-Pozo *et al*. (1986), Harzer & Haschke (1989), Renner *et al*. (1989), Fox & McSweeney (1998), Walstra *et al*. (1999) and Gopal & Gill (2000).

Table 21.4 Essential and semi-essential amino acids in human milk.

Amino acid	mg per 100 kJ	mg per 100 kcal
Cysteine	9	38
Histidine	10	40
Isoleucine	22	90
Leucine	40	166
Lysine	27	113
Methionine	5	23
Phenylalanine	20	83
Threonine	18	77
Tryptophan	8	32
Tyrosine	18	76
Valine	21	88

Source: reproduced from Commission of the European Communities Directive 2006/141/EC on infant formulae and follow-on formulae. © European Union, http://eur-lex.europa.eu/

When designing infant formulae, differences in total protein content, whey protein–casein ratio and amino acid profiles of human milk and bovine milk are key factors that must be taken into consideration. Although several early infant formulae featured lower protein levels than in bovine milk, milk-based formulae remained casein dominant until 1961, when a formula with a whey protein–casein ratio similar to that of human milk (60 : 40) was developed (Lien, 2003). This was enabled as the technology to demineralise whey was developed for the specific purpose of including whey protein in infant formulae (Clark *et al*., 1965). Prior to this, whey was unsuitable for use in infant formulae due to its high mineral content and the contribution of these minerals to instability during heat processing. By combining skimmed milk solids and demineralised whey, a formula containing 1.5 g protein per 100 mL and a 60 : 40 whey protein–casein ratio was produced (Lien, 2003). Later, ultrafiltration of whey was another important development as it led to the use of whey protein concentrates (WPCs) in infant formulae manufacture. At 15 g/L, the protein content of the whey-dominant formula remains higher than that of human milk (9–11 g/L). This is in order to compensate for the lower levels of certain essential amino acids, specifically tryptophan and cysteine, in bovine milk compared to human milk (Lien, 2003). Research is ongoing to improve the protein compliment of the whey protein dominant infant formula. This research is centred on reducing the overall protein content, matching more closely the proportions of the individual whey proteins and caseins in human milk while at the same time ensuring that the essential amino acid content meets regulatory guidelines and standards. In order to reduce the overall protein content of infant formulae, it is necessary to use ingredients rich in the essential amino acids or fortify with free amino acids.

Recent developments in milk protein fractionation and isolation technologies have led to the availability of novel ingredients for use in infant formulae. Many of these fractionation techniques were reviewed by O'Regan *et al*. (2009). An ingredient particularly suitable for inclusion in infant formulae is an α-lactalbumin (ALA)-enriched whey protein fraction. It is possible to produce an ALA-enriched fraction by subjecting whey to mild heat treatment under controlled pH and ionic conditions that cause the reversible precipitation of an ALA-enriched fraction, and the subsequent separation of the precipitate from the mother liquor. The precipitate may be resolubilised by water addition and pH adjustment and then dried.

There has been a considerable amount of research conducted on isolation technologies and the interested reader is referred to Pearce (1983, 1987), Maubois *et al*. (1987), de Wit and Bronts (1994), Bramoud *et al*.(1997), Mehra and Kelly (2004), Cheang and Zydney (2004), da Fonseca *et al*. (2005) and Bhattacharjee *et al*. (2006) for further details. The ALA-enriched whey protein fraction has many desirable formulation attributes. ALA is the dominant whey protein in human milk (β-lactoglobulin is the dominant whey protein in bovine milk and is absent in human milk), so the enrichment of formula with ALA brings the protein profile of human milk and infant formula a step closer. It contains high levels of tryptophan and other essential amino acids, thus facilitating the design of a lower protein formula (13–14 g/L) than when using demineralised whey (Lien, 2003; Kuhlman *et al*., 2005). A study by Davis and Harris (2005) found that plasma essential amino acid levels that resulted from feeding an experimental infant formula, containing protein at 14 g/L and enriched in ALA, were generally higher than those from feeding a control formula containing protein at 15 g/L but not enriched in ALA. Furthermore, there were fewer feeding-related withdrawals and improved gastrointestinal tolerance associated with the experimental formula than the control formula owing to the apparent prebiotic effect of the ALA fraction (Bettler & Kullen, 2007; Kullen *et al*., 2009).

Research studies appear to support the enrichment of infant formulae with other whey proteins especially lactoferrin and secretory IgA (sIgA). These proteins, rich in human milk, are reported to play important physiological roles in the infant's defence mechanism. Lactoferrin, the second most abundant protein in human milk (~2 g/L), exists only in trace quantities in bovine milk (~0.01 g/L). In bovine milk, following an initial decrease in lactoferrin levels after birth, levels increase slightly throughout lactation and increase

significantly throughout the dry period or during mastitic infection. Such an increase does not occur in human milk (Jenness, 1982). Lactoferrin is positively charged at neutral pH, whereas the other whey proteins (apart from lactoperoxidase) are negatively charged. Thus, it is possible to use cation exchange chromatography to isolate lactoferrin-enriched fractions from whey (Paul *et al*., 1980; Prieels & Pfeiffer, 1986; Burling, 1989). Lactoferrin has also been separated from whey by precipitation using ammonium sulphate followed by separation on carboxymethylcellulose(Yoshida & Xiuyun, 1991) and also by gel permeation methods (Alwan Al-Mashikhi & Nakai, 1987). The ability of lactoferrin to bind iron strongly is important in iron absorption and protection of the neonate against enteric infections. The level of iron saturation by lactoferrin in human milk is relatively low at 6–8% compared with bovine milk with a saturation of 20–30%. Lactoferrin has been associated with a vast array of biological activities, including enhancing the bioavailability of iron, inhibition of iron-mediated oxidation reactions, regulation of inflammatory reactions, modulation of the intestinal flora and its bacteriostatic and bactericidal activity. While many of the biological activities associated with lactoferrin have been demonstrated in animal models, some infant studies have been conducted showing that supplementation with lactoferrin has been shown to reduce the incidence of late-onset sepsis in very low-birthweight neonates (Manzoni *et al*., 2009); a pilot double-blind study on infant formula supplemented with lactoferrin (850 mg/L) showed significantly fewer lower respiratory tract illnesses and higher haematocrits (King *et al*., 2007).

Another protein fraction of keen interest to infant formula manufacturers is the immunoglobulin family. Human milk contains three subclasses of immunoglobulin with sIgA being the principal one (representing 90% of immunoglobulin protein). Immunoglobulin proteins are involved in the protection against infection and are present in mammary secretions. There is considerable interest in the use of immunoglobulins to enrich formulae for preterm infants. Milk enriched in sIgA has been produced by cows treated with immunogens that stimulate sIgA production (Barrio *et al*., 2003). A fraction enriched in immunoglobulins was prepared by ultrafiltration and diafiltration of milk from immunised cows (Hilpert, 1984). A combination of ultrafiltration and ion exchange chromatography has been used to separate immunoglobulins from lactoferrin (Bottomley, 1989) and from colostrum or milk from hyperimmunised cows (Taniguchi *et al*., 1990). Metal chelate interaction chromatography (MCIC), a separation technique based on the interaction of exposed histidine, cysteine and tryptophan side chains with immobilised copper ions, was used to isolate fractions enriched in immunoglobulins (75–95% purity) from cheese whey (Li-Chan *et al*., 1990).

Compared with recent developments in bringing the whey protein composition and complement of infant formula closer to those of human milk, less research has focused on the caseins. Human milk casein is primarily composed of β-casein with about 15% κ-casein and low levels of α_s-casein. Several techniques for fractionating whole bovine casein into the individual caseins were reviewed by O'Regan *et al*.(2009). Essentially all methods employed for fractionating caseins are based on the association characteristics of the individual caseins. In a micellar casein system such as milk and in a dispersion of calcium or sodium caseinate, the individual casein molecules associate via a range of forces, including hydrophobic interactions. The strength of hydrophobic interactions is temperature dependent and at low temperatures (<5°C) when hydrophobic interactions are weaker, the β-casein molecules dissociate from α_s-/κ-casein complexes and exist in solution as monomers (O'Regan *et al*., 2009). β-Casein fractions have been isolated by exploitation of its dissociation properties at low temperatures (4°C), under which conditions the β-casein remains soluble, while the remaining caseins can be precipitated by rennet and/or calcium and can be subsequently separated by centrifugation (Payens & van Markwijk, 1963; Huppertz *et al*., 2006). Law and Leaver (2004) described a method of selective precipitation of all four caseins from skimmed milk or caseinate. Techniques to isolate β-casein that have potential to be scaled up to industrial level include those based on ultrafiltration (Murphy & Fox, 1991) and microfiltration (Famelart *et al*., 1989; Turhan *et al*., 2003). The recent industrial advancements in manufacturing high-purity casein fractions are believed to further evolve the humanisation of infant formula. However, there is limited information in the literature on the use of specifically designed casein fractions in infant formulae. A rat growth study conducted by van Dael *et al*. (2005), where infant formula enriched in β-casein was used as a substitute for casein, showed that the protein nutritional quality, as determined by the protein efficiency ratio, net protein utilisation, biological value and protein digestibility, and the mineral absorption and retention balance was unaffected when casein was replaced with β-casein.

Casein-dominant formulae are generally perceived to be more satisfying for hungry infants than whey-dominant formula. Casein-dominant formulae commonly feature a casein–whey protein ratio of 80 : 20 as found in bovine milk. The protein is generally provided by skimmed milk solids and there is no added whey protein. Caseinates and milk protein isolates are also used in low-lactose formulae for lactose-intolerant infants, while selected caseinates are used in the production of infant foods where a specific mineral balance is required, for example low-sodium infant formulae for children with specific renal problems.

Other minor proteins of particular interest to the infant formula industry have recently become commercially available; these include milk basic protein, lactoperoxidase, osteopontin, caseinoglycomacropeptide and milk fat globule membrane protein fractions. Milk protein-derived biologically active peptides have received a lot of attention recently and the trend is likely to continue. Biologically active peptides can be generated *in vivo* during gastrointestinal digestion of milk protein or released *in vitro* on hydrolysis of milk proteins; these biologically active peptides include angiotensin-converting enzyme (ACE) inhibitors, opioid agonists or antagonists, and antithrombotic, antimicrobial, immunomodulatory and anxiolytic peptides. While the concept of adding such biologically active peptides is certainly attractive, the challenge lies in the development of commercially feasible fractionation processes that will generate sufficient and sustainable volumes of these peptides at cost-effective prices.

Overall, the procurement of substantial volumes of novel ingredients at cost-effective prices is an ongoing challenge to the producers of infant formulae (Jost *et al*., 1999; O'Callaghan *et al*., 2011).

Table 21.5 Principal fatty acids (wt% of total) in milk triglycerides or total lipids from human and bovine milk.

Fatty acid	Bovine	Human
C4:0 (butyric acid)	3.3	—
C6:0 (caproic acid)	1.6	—
C8:0 (caprylic acid)	1.3	—
C10:0 (capric acid)	3.0	1.3
C12:0 (lauric acid)	3.1	3.1
C14:0 (myristic acid)	9.5	5.1
C16:0 (palmitic acid)	26.3	10.2
C16:1 (palmitoleic acid)	2.3	5.7
C18:0 (stearic acid)	14.6	5.9
C18:1 (oleic acid)	29.8	46.4
C18:2 (linoleic acid)	2.4	13
C18:3 (linolenic acid)	0.8	1.4
C20:4 (arachidonic acid)	—	0.5
C22:6 (docosahexaenoic acid)	—	0.32

Source: based on data from Christie (1995).

21.5.2.3 Lipids

Lipids are the main energy source in human milk and infant formulae, providing the infant with 40–50% of its total energy intake. The lower limit for fat in infant formulae is set at 3.3 g/100 kcal by the major regulatory bodies (Table 21.1). Minimum levels have been set for linoleic acid (C18:2) and linolenic acid (C18:3) as they cannot be synthesised by infants and must be provided in the diet. Upper levels have been set for certain fatty acids that are not abundant in human milk, namely lauric (C12:0), myristic (C14:0) and erucic (C22:1) acids. Formulation of the lipid component involves devising a fat blend that satisfies the regulations and which has a fatty acid profile similar to that of human milk fat. Bovine milk fat, on its own, is largely unsuitable for first-age formula as it contains higher levels of the short-chain fatty acids (C4:0–C8:0) and lower levels of unsaturated (C18:1, C18:2, C18:3) and polyunsaturated (C20:4, C22:6) fatty acids than human milk (Table 21.5). As early as 1919, an infant formula designated and labelled SMA ('simulated milk adapted') contained a physiological fat blend derived from animal and vegetable fats (Gerstenberger *et al*., 1919). Today the fat in most commercial infant formulae consists of blends of vegetable oils such as palm, coconut, soybean, high oleic safflower and sunflower oils that are blended in ratios to provide a similar fatty acid profile to that found in human milk. The fatty acid compositions of the permitted vegetable oils are outlined in Table 21.6. It had been assumed that infants could synthesise adequate amounts of arachidonic acid (ARA, C20:4) and docosahexaenoic acid (DHA, C22:6) from their respective precursors linoleic acid and linolenic acid. However, it became apparent that infants benefited from formulae that provided both preformed ARA and DHA (Carver, 2003).

Although fish oil is a rich source of DHA, unfractionated fish oil is not suitable for addition to infant formula as it contains eicosapentaenoic acid (C20:5), a fatty acid which is not present in human milk and is known to be antagonistic to the functions of ARA (Carlson *et al*., 1992). The technology to isolate significant quantities of ARA and DHA from microalgal and fungal oils, egg yolk-derived lipids and marine oils has been developed and commercialised. There are data linking dietary long-chain polyunsaturated fatty acid intake with beneficial effects on neurodevelopmental maturation in term infants. Paediatric experts recommend that infant formulae for term infants contain at least 0.2% of the fatty acids as DHA and 0.35% as ARA (Koletzko *et al*., 2001). In 2009, the European Food Safety Authority determined that in order to bear a claim linking DHA with the visual development of infants, a formula should contain at least 0.3% of the total fatty acids as DHA.

If infants are fed fat blends with palmitic acid esterified at the *sn*-1 and *sn*-3 positions, the resulting free fatty acids may form poorly absorbed calcium soaps (Nelson *et al*., 1996). Recently, a novel triacylglycerol containing palmitic acid at the *sn*-2 position has been developed by the interesterification of tripalmitin with fatty acids from high oleic sunflower oil or soybean oil in the presence of a 1,3-specific

Table 21.6 Typical fatty acid composition (wt% of total) of human milk and vegetable oils typically used in infant formulae.

Carbon atom: double bonds	Human milk	Coconut oil	Palm oil	Palm kernel oil	Safflower oil	Safflower oil (high oleic)	Soybean oil	Sunflower oil
4:0 (butyric)	—	—	—		—	—	—	—
6:0 (caproic)	—	0.5	—	0.3	—	—	—	—
8:0 (caprylic)	—	8	—	3.9	—	—	—	—
10:0 (capric)	1.3	6.4	—	4.0	—	—	—	—
12:0 (lauric)	3.1	48.5	0.3	49.6	—	—	—	0.5
14:0 (myristic)	5.1	17.6	1.1	16.0	0.1	0.1	0.1	0.2
16:0 (palmitic)	10.2	8.4	45.1	8.0	6.5	5.5	11.0	6.8
16:1 (palmitoleic)	5.7	—	0.1	—	—	0.1	0.1	0.1
18:0 (stearic)	5.9	2.5	4.7	2.4	2.4	2.2	4.0	4.7
18:1 (oleic)	46.4	6.5	38.8	13.7	13.1	79.7	23.4	18.6
18:2 (linoleic)	13	1.5	9.4	2.0	77.7	12.0	53.2	68.2
18:3 (linolenic)	1.4	—	0.3	—	—	0.2	7.8	0.5
20:0 (arachidic)	—	0.1	0.2	0.1	0.2	0.2	0.3	0.4
20:4 (arachidonic)	0.5	—	—	—	—	—	—	—
22:0 (behenic)	—	—	—	—	—	—	—	—
22:1 (erucic)	—	—	—	—	—	—	—	—
22:2 (docosadienoic)	—	—	—	—	—	—	—	—
22:6 (docosahexaenoic)	0.32	—	—	—	—	—	—	—

Sources: based on data from Christie (1995) and Orthoefer (1996).

enzyme (Gunstone, 2001). This triacylglycerol is structurally similar to triacylglycerols found in human milk fat and is specially designed for use in infant formulae to mimic the fatty acid distribution of human milk (Osborn & Akoh, 2002). From research based on an infant feeding study, Carnielli *et al*.(1996) concluded that dietary triglycerides containing palmitic acid predominantly at the *sn*-2 position, as in human milk, have significant beneficial effects on the intestinal absorption of fat and calcium.

21.5.2.4 Carbohydrate

Carbohydrate is essential for the infant as an energy source and a diet devoid of carbohydrate eventually leads to hypoglycaemia. A minimum level of carbohydrate in infant formulae is not specified by the United States FDA (1980) or the Codex Alimentarius Commission (1981) but the Commission of the European Communities (2006) specifies a lower limit of 9 g/100 kcal (Table 21.1). In the case of dairy-based formulae, the carbohydrate is primarily lactose. Other carbohydrate sources (maltose, sucrose, corn syrup solids, maltodextrins, glucose syrup, pre-cooked starch and gelatinised starch) may be used.

There appears to be scientific evidence to support the enrichment of infant formulae with complex oligosaccharides similar to those found in human milk. The reader is referred to Urashima *et al*. (2011) for a more detailed overview of the composition and structure of oligosaccharides found in human milk. The inclusion of these oligosaccharides would bring the composition of infant formulae closer to human milk. Furthermore, these components appear to play important biological roles in brain development and the prevention of enteric infections (Kunz & Rudloff, 1993; Klein *et al*., 2000) and thus infants fed oligosaccharide-enriched formulae may have similar physiological outcomes to breast-fed infants. These oligosaccharides may also have prebiotic effects, thus favouring the growth of bifidobacteria and lactobacilli in the infant's digestive tract.

However, isolating large volumes of oligosaccharides, which are present at low levels in bovine milk, is currently a technical challenge to the dairy industry. Sialyllactose, the main oligosaccharide found in bovine milk, is contained in the non-protein nitrogen fraction of whey. Sarney *et al*. (2000) described a laboratory-scale process to recover the oligosaccharides from human milk. In this process, the proteins in skimmed milk were precipitated and the remaining filtrate was treated with β-galactosidase to hydrolyse the lactose. The resultant mixture of enzyme, glucose, galactose and oligosaccharides was ultrafiltrated (molecular mass cut-off, 10 kDa) to remove the enzyme and then nanofiltrated

where the oligosaccharides were concentrated in the retentate. La Ferla *et al*. (2002) describe the synthesis of the disaccharides Galβ(1→3)GlcNAc and Galβ(1→4)GlcNAc by chemical and enzymatic methods. These compounds could serve as building blocks to prepare complex oligosaccharides such as those found in human milk. The synthesis of 3′- and 6′-sialyllactose, also by chemical and enzymatic methods, was described by Rencurosi *et al*. (2002).

21.5.2.5 Minerals

The level of total ash in bovine milk (0.7% w/w) is considerably higher than in human milk (0.2% w/w). In general, bovine milk contains higher levels of the major minerals (Na, K, Cl, Ca, Mg and P) than human milk but the levels of the minor and trace elements are relatively similar (Table 21.7).

In bovine milk, the relatively high levels of the electrolytes (Na, Cl, K and P) and protein result in a high potential renal solute load (PRSL). The PRSL is closely related to the amount of water that is involved in the excretion by the kidneys. The PRSL of human milk is 14 mosmol/L compared with 46 mosmol/L for bovine milk (Fomon & Ziegler, 1993, 1999). It was not until the development of demineralisation that whey could be used in infant formula production due to its high ash content. In the case of certain minerals, where the inherent level from the protein and carbohydrate sources is less than the declared level, fortification using permitted salts is required. An in-depth understanding of how these salts influence the processing characteristics and shelf-life stability of the formula is required in order to make an appropriate selection. The impact on the pH of the formula is one consideration and certain salts, for example calcium chloride and the sodium and potassium salts of citric acid, are known to influence the heat stability of milk protein systems. Other salts, for example calcium phosphate, are insoluble and thus sediment over time. Ferrous sulphate is a potent pro-oxidant thus accelerating lipid oxidation.

Human milk contains higher levels of certain minerals, including Fe, Cu, Co and Cr, than bovine milk (Table 21.7). Selenium (Se), an essential trace mineral, is present in human milk at about 15–20 μg/L compared with about 2–13 μg/L in unsupplemented bovine milk-based infant formulae (Carver, 2003); Se is now routinely added to infant formulae.

Table 21.7 Mineral composition (mg or μg/L) of mature human and bovine milks.

	Human		Bovine	
Constituent	**Mean**	**Range**	**Mean**	**Range**
Sodium (mg)	150	110–200	500	350–900
Potassium (mg)	600	570–620	1500	1100–1700
Chloride (mg)	430	350–550	950	900–1100
Calcium (mg)	350	320–360	1200	1100–1300
Magnesium (mg)	28	26–30	120	90–140
Phosphorus (mg)	145	140–150	950	900–1000
Iron (μg)	760	620–930	500	300–600
Zinc (μg)	2950	2600–3300	3500	2000–6000
Copper (μg)	390	370–430	200	100–600
Manganese (μg)	12	7–15	30	20–50
Iodine (μg)	70	20–120	260	—
Flourine (μg)	77	21–155	—	30–220
Selenium (μg)	14	8–19	—	5–67
Cobalt (μg)	12	1–27	1	0.5–1.3
Chromium (μg)	40	6–100	10	8–13
Molybdenum (μg)	8	4–16	73	18–120
Nickel (μg)	25	8–85	25	0–50
Silicon (μg)	700	150–1200	2600	750–7000
Vanadium (μg)	7	Trace–15	—	Trace–310
Tin (μg)	—	—	170	40–500
Arsenic (μg)	50	—	45	20–60

Source: reproduced from Flynn & Power (1985), with permission of Elsevier.

21.5.2.6 Vitamins

Vitamins are organic molecules essential for the biological processes of higher organisms but which cannot be synthesised by these organisms. They are frequently differentiated by their solubility in fat and water. The fat-soluble vitamins are associated with the fat fraction of milk, whereas the water-soluble vitamins are associated with the non-fat aqueous fraction or whey. The water-soluble vitamins and vitamin K act as coenzymes. Vitamin A is important for vision, vitamin D functions as a hormone and vitamin E is an antioxidant. Renner *et al*. (1989) and Fox and McSweeney (1998) have reviewed the vitamins of bovine milk while Harzer and Haschke (1989) reviewed the vitamins present in human milk. Human and bovine milk contain relatively similar levels of vitamins (Table 21.8). The chemical forms of many vitamins are different from those in human milk. In human milk, vitamin E is present as various tocopherols, whereas pure α-tocopherol is typically used in infant formulae. A commercial preparation of mixed carotenoids isolated from vegetable oils is used to mimic the complex carotenoid profile of human milk (O'Callaghan *et al*., 2011).

Table 21.8 Levels (mg or μg/100 g) of vitamins in human milk and in whole and skimmed bovine milk.

Vitamin	Human	Bovine (whole)	Bovine (skimmed)
Fat-soluble vitamins			
Vitamin A (retinol) (μg)	58	52	1
Carotene (μg)	24	31	Trace
Vitamin D (μg)	0.4	0.03	Trace
Vitamin E (μg)	0.34	0.09	Trace
Vitamin K (μg)	0.2	0.4	Trace
Water-soluble vitamins			
Vitamin B_1 (thiamine) (mg)	0.02	0.04	0.04
Vitamin B_2 (riboflavin) (mg)	0.03	0.17	0.18
Vitamin B_6 (pyridoxine) (mg)	0.01	0.06	0.06
Vitamin B_{12} (cyanocobalamin) (mg)	Trace	0.4	0.4
Niacin (mg)	0.2	0.1	0.1
Folic acid (μg)	5.0	6	6
Pantothenic acid (mg)	0.25	0.35	0.32
Biotin (μg)	0.7	1.9	2.0
Vitamin C (mg)	4.0	1	1

Source: based on data from Holland *et al*. (1991).

21.5.2.7 Probiotics, prebiotics and synbiotics

The intestinal flora of breast-fed and formula-fed infants is quite different. Bifidobacteria are the dominant bacteria in breast-fed infants whereas in formula-fed infants similar levels of *Bacteroides* spp. and bifidobacteria are found (Harmsen *et al*., 2000). The addition of probiotic bacteria, such as *Bifidobacterium lactis*, *Streptococcus thermophilus* and *Lactobacillus rhamnosus* GG among others, to infant formulae has produced some interesting outcomes in the treatment and prevention of infectious diarrhoea and allergy (Alles *et al*., 2004). Saavedra *et al*. (2004) found that there was a reduction in the reporting of colic or irritability and a lower frequency of antibiotic use when infants were fed an infant formula supplemented with *B. lactis* and *S. thermophilus* compared with an unsupplemented formula. Wilschanski *et al*. (2005) reported that supplementation of formula with probiotics (*Bifidobacterium infantis*, *Bifidobacterium bifidus*, *S. thermophilus*) was effective in reducing both the incidence and severity of necrotising enterocolitis in low-birthweight infants. Dehydrated prebiotic cultures in a suitable carrier system such as maltodextrin are commercially available for use in infant formulae production.

The dominance of bifidobacteria in the intestinal flora of breast-fed infants has been attributed to the oligosaccharides in human milk. Oligomers of simple sugars, for example fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), are also prebiotic (Vandenplas, 2002) and these complex carbohydrates have been added to infant formula (Alles *et al*., 2004) even though they have different chemical structures from the oligosaccharides found in human milk (O'Callaghan *et al*., 2011). Fuentes *et al*. (2005) found that feeding a FOS/GOS-supplemented infant formula to healthy infants produced some outcomes similar to breast-fed infants, such as an increase in the proportion of bifidobacteria in the stools, low faecal pH and soft stool consistency.

Synbiotics are combinations of prebiotics and probiotics. Fisberg *et al*. (2002) found that preschool children on a formula supplemented with synbiotics (*Bifidobacterium* spp., *Lactobacillus acidophilus* and FOS) experienced less incidences of illness compared to children on an unsupplemented formula.

21.5.2.8 Other nutrients

Taurine, a sulphur-containing β-amino acid with a sulphonic acid group, is found in higher concentrations in human milk than in bovine milk. In 1981, supplementation of infant formulae with taurine began in the EU and since the US Food and Drug Administration gave approval for supplementation in 1984, it is now a routinely added nutrient. Taurine is purported to play a role in fat absorption and the development of the brain and retina (Heird, 2004).

Nucleotides (adenosine, cytidine, guanosine, uridine and inosine monophosphates) account for 2–5% of the non-protein nitrogen fraction of human milk (Thorell *et al*., 1996), but lower levels are present in bovine milk and unsupplemented infant formulae. As nucleotides are believed to play key roles in many biological processes, including immune function (Carver, 2003), they may be added to infant formula.

Choline serves as a precursor for the biosynthesis of phospholipids and may be important for brain development (Zeisel, 2004). Bovine milk and infant formulae have similar levels of choline to human milk (Raiten *et al*., 1998). A minimum choline limit of 7 mg/100 kcal was set by the US Food and Drug Administration (1980) and the Commission of the European Communities (2006) (Table 21.1). If the raw materials do not provide sufficient choline, fortification may be provided using a choline salt such as choline chloride.

It is not certain whether inositol is an essential nutrient; although it is synthesised endogenously, several clinical

conditions are known to impair synthesis (Raiten *et al*., 1998). Human milk contains about 149 mg/L (22 mg/100 kcal). A minimum inositol limit of 4 mg/100 kcal was set by the US Food and Drug Administration (1980) and later by the Commission of the European Communities (2006) (Table 21.1). Fortification may be provided if the unsupplemented levels are less than this minimum level.

Lutein, a xanthophyll and a member of the carotenoid family of compounds, is a structural component of the eye and studies suggest that it may help provide protection against oxidative and 'blue light' damage (Capeding *et al*., 2010). Humans cannot synthesise carotenoids so blood and tissue levels depend on dietary consumption. It is present in human milk at low levels (~25 μg/L) and has recently been added to infant formula (Bettler *et al*., 2010). Exploratory research suggests that DHA and lutein supplementation may have cognitive benefit for older adults (Johnson *et al*., 2008).

21.5.2.9 Processing aids and food additives

Processing aids and food additives may be required in certain instances to manufacture products with an acceptable appearance and sensory characteristics. These ingredients include hydrocolloids (thickening agents and stabilisers), emulsifiers, pH-adjusting agents and antioxidants.

The formation of a stable oil-in-water emulsion is a common prerequisite of both the powder and liquid production processes in the manufacture of infant formulae. Scientific committees advise that the number of processing aids used in infant nutritional products is kept to a minimum. The producers of infant formula take into account the considerable amount of safety studies and supporting documentation required to prove safety of an emulsifier in infant nutritional products and adhere to the strict regulations which specify the upper release limits for such food additives (McSweeney, 2008). In regular infant and follow-on formula, only limited levels of two emulsifiers, lecithin (E322) and monodiglycerides (E472a), are permitted for use in these products. However, in the case of speciality nutritional products containing either hydrolysed proteins, peptides or free amino acids, the use of non-protein emulsifiers is often essential to stabilise the emulsion. This is reflected in a more extensive list of emulsifiers permitted in these speciality products such as citric acid esters of monoglycerides (CITREM,E472c), sucrose esters of fatty acids (E1450) and 'mono- and di-acetylated tartaric acid esters of mono- and diglycerides' (DATEM, E472e).

As with emulsifiers, hydrocolloids are regulated as food additives. Starch may be used as a source of carbohydrate and is permitted up to a maximum level of 0.2 g/L and 30% of the total carbohydrate in infant formula (McSweeney, 2008). Starches and gums may be chemically or enzymatically modified to insert a lipophilic group. For example, alginic acid may be esterified with propylene glycol to yield propylene glycol alginate (E405). Other regulatory agencies such as the Codex Alimentarius permit addition of modified starches including distarch phosphate (E1412), acetylated distarch phosphate (E1414), phosphated distarch phosphate (E1413) and hydroxyl propyl starch (E1400) to first-age infant formula.

21.5.3 Specialised first-age infant formulae

Special diets have been developed for infants who fail to thrive on regular first-age formulae or have special nutritional requirements including metabolic or digestive disorders. Some of these formulae fall under the general heading 'food for special medical purposes' (Commission of the European Communities, 1999). Formulae based on soy protein are available for infants who display intolerance to milk protein and are typically fed to infants experiencing non-specific gastrointestinal problems. The protein source, soy protein isolate, typically contains 80–90% protein. If the carbohydrate source is other than lactose, the formulae may also be suitable for infants who display lactose intolerance. The protein level in soy formula is usually higher than that of milk-based formula, because of differences in protein digestibility and amino acid composition. The other ingredients are similar to those used in bovine milk-based formulae. As carnitine is generally absent from soy protein sources, soy-based formulae are fortified with L-carnitine.

Dairy-derived formulae may not be suitable for some patients because of lactose sensitivity or intolerance. This intolerance is often due to the patient not having sufficient β-galactosidase in the small intestine to digest lactose. This maldigestion of lactose inevitably leads to intestinal disorders such as flatulence, bloating and abdominal pain leading to discomfort and diarrhoea. It is also possible to formulate milk protein-based formulae for infants who are lactose intolerant but who can tolerate milk protein. These formulations are based on milk protein and whey protein, in the form of concentrates or isolates, from which the lactose has been removed by membrane filtration or enzymatic hydrolysis.

Increasing incidences of intolerance and allergic diseases in developed countries highlights the importance and requirement for hypoallergenic infant nutrition. Formulae based on hydrolysed protein are available for infants with intact milk protein allergy and infants with mild to moderate milk protein intolerance. These products can be broadly classified according to the degree of protein hydrolysis as 'partially' or 'extensively' hydrolysed protein.

Extensively hydrolysed proteins (casein, whey protein or soy protein hydrolysates) are used in products for the

dietary management of cows' milk allergy and are generally recommended for atopic infants who have a hereditary predisposition towards developing certain hypersensitivity reactions upon exposure to specific antigens. The production of these ingredients generally involves enzymatic hydrolysis of protein to peptides of low molecular weight followed by ultrafiltration to remove unhydrolysed protein and large polypeptides. Partially hydrolysed proteins are used in products referred to as 'comfort' or 'easy-to-digest' formulae; these proteins have been shown to have improved absorption in gastrointestinal digestive system of infants. These formulae are often targeted towards otherwise healthy term infants who have subclinical discomfort, including flatulence, fussiness, bloating and other gastrointestinal disorders. The production of these ingredients is similar to that for extensively hydrolysed protein, but generally involves a shorter enzymatic hydrolysis phase and the unhydrolysed protein and large polypeptides are retained in the finished hydrolysate.

Casein hydrolysates are used in specialised formulations for premature infants and in formulae for infants suffering from various intestinal disorders; casein hydrolysate-based formulae, low in phenylalanine, have been proposed for feeding infants with phenylketonuria. The absence of phenylalanine, tyrosine, tryptophan and cysteine residues in κ-casein glycomacropeptide (GMP) make it a possible stable source of nutrition for phenylketonuria-sensitive patients. The glycomacropeptide of κ-casein has also been shown to modulate the gut microflora as the carbohydrate moieties (sialic acid) promote the growth of bifidobacteria while also preventing the attachment of bacteria to the gut. Non-glycosylated forms of GMP, referred to as caseinomacropeptide, are reported to inhibit gastric secretions and slow down stomach muscle contractions.

Stringent regulations accompany claims for a hypoallergenic formula. In Europe, a 99% reduction in immunoreactive protein and a positive animal sensation study must be demonstrated prior to claiming a reduction of risk to milk protein allergy. Therefore partially hydrolysed formulae cannot be prescribed for atopic infants. Extensively hydrolysed whey proteins and free amino acids have been used in hypoallergenic, peptide-based formulae as they have been proved clinically efficacious for management of atopic infants. Only pure amino acid mixtures are considered non-allergenic, and elemental diets containing free amino acids are prescribed for infants with highly allergic conditions.

Infants prone to the involuntary passage of gastric contents into the oesophagus, a condition known as gastro-oesophageal reflux (GOR), may be fed formulae that have the capacity to become viscous after ingestion. Permitted hydrocolloids such as starch or locust bean gum are used in specially formulated anti-regurgitation formulae. The use of anti-regurgitation formulae has been shown to reduce the incidence of reflux episodes in GOR-prone infants.

Some infants, for example those classed as small for gestational age or those who fail to thrive, have increased nutritional requirements. High-caloric or nutrient-dense formulae, typically 900 kcal (3800 kJ)/L, are prescribed for infants with increased nutritional requirements.

21.5.4 Formulae for low-birthweight and premature infants

Most pregnancies last around 40 weeks. Infants born before 37 completed weeks of pregnancy are called premature or preterm. Newborn infants that weigh less than 2500 g are conventionally defined as low birthweight. These infants require a special diet in order to survive and achieve the growth and development rates of normal infants. In general, low-birthweight infants have smaller nutrient stores and more underdeveloped digestion and absorption capabilities than full-term infants. In the hospital, premature and low-birthweight infants are fed fortified breast milk or, in circumstances where that is not possible, a specially designed formula for low-birthweight infants is prescribed. Generally, the milk of a mother that has delivered a preterm infant is more nutrient dense than regular term milk but it may not fully support the infant's rapid growth rate so it is normal practice to enrich the milk with a nutritional preparation known as 'human milk fortifier'. The fortifier supplements human milk with protein, minerals, in particular calcium, together with electrolytes and vitamins. Formulae for low-birthweight infants contain greater amounts of protein, vitamins, minerals and calories than standard infant formulae to address their high nutrient needs. Low birthweight formulae are based on guidelines recommended by several expert groups such as the Association of the Food Industries for Particular Nutritional Uses of the European Union, the European Society for Paediatric Gastroenterology Hepatology and Nutrition, the Life Sciences Research Office (LSRO) of the American Society for Nutritional Sciences, the American Academy of Pediatrics Committee on Nutrition, and Tsang *et al.* (2005). The guiding principle is that the low-birthweight formula should provide the nutrients necessary to achieve postnatal growth rates and nutrient accretion that matches those of a normal fetus during the same period (Carver, 2003). A 'post-discharge formula' is available for the infant transitioning from the low-birthweight formula to standard formula. This is a nutrient-enriched formula generally intermediate in composition between preterm and term formula (Carver *et al.*, 2001;Lucas *et al.*, 2001). It supports rapid growth and prevents nutrient depletion without providing excess nutrients.

21.5.5 Follow-on formulae

Follow-on formulae are intended as nutritional supplements for infants older than 4–6 months. These formulae are the main liquid component of a progressively diversified diet. It is normal at this stage for the infant to be gradually introduced to other foods such as cereals, fruit, vegetables and eventually meat and eggs.

A summary of the nutrient limits in follow-on infant formulae set out in the European Directives and the Codex Alimentarius is given in Table 21.9. Follow-on formulae generally contain higher contents of protein and carbohydrate, but less fat than standard first-age formulae. The types of fat, protein and carbohydrate present in follow-on infant formula are similar to those of standard first-age infant formulae. Follow-on formulae are designed to provide the infant with a superior nutritional source than bovine milk. Thus, these formulae have increased levels of iron, zinc, vitamin C and fat-soluble vitamins and reduced levels of fat compared with bovine milk. Other nutrients such as nucleotides, ARA and DHA may also be added.

21.5.6 Growing-up milks

The growing-up, or toddler, years are critical periods of physical and emotional development and are characterised by variable dietary patterns. Specially formulated milks referred to as 'growing-up milks' are designed for toddlers. The composition of these products is typically guided by authoritative guidelines for nutritional intakes such as Dietary Reference Intake (DRI) from the Institute of Medicine of the US National Academy of Sciences. These milks provide protein, iron, calcium and other essential vitamins and minerals for optimal growth and development. The sensory attributes of these formulae must be appealing and vanilla, chocolate and a range of fruit flavourings are commonly added to enhance the taste and aroma of these products. Some children aged 1 year and above are fussy or 'picky' eaters and, recently, specially designed formulae for these children have been successfully introduced. These formulae generally contain the nutritional components found in the seven major food categories (grains, vegetables, fruits, fish, meat, eggs and milk) and are specifically supplemented with 'at-risk' nutrients that fussy eaters might be lacking. The formula serves as a nutritional safety net when the child's diet might not include all food groups.

21.5.7 Formulae for pregnant and lactating women

In recent years, nutritional preparations for pregnant and lactating women have become available. These products are based on the concept that a pregnant or lactating woman is constantly 'feeding' her child from the nutrients in her own body, thus depleting stores of vitamins and minerals (Lawrence & Lawrence, 2011b). A wide variety of these products are available in different presentations such as multivitamin and mineral tablets or capsules, or beverages enriched in these nutrients. DHA is another common nutrient in these products. Supplementation of the mother's diet with a source of DHA increases breast milk DHA levels (Helland *et al*., 2003).

21.6 PROCESSING AND MANUFACTURE OF FORMULAE FOR INFANTS AND YOUNG CHILDREN

Processes used in the manufacture of formulae for infants and young children are based on the concept that the products must be nutritionally adequate and microbiologically safe to consume. Thus, steps that eliminate or restrict microbiological growth are central to production processes. The processing technology for each specific formula is proprietary to the manufacturer but, in general, it involves the preservation of an oil-in-water (o/w) emulsion by dehydration in the case of powder products, or sterilisation in the case of ready-to-feed or concentrated liquid products. Powdered infant formula may be produced using various processes, such as dry blending dehydrated ingredients to constitute a uniform formula or hydrating and wet-mixing a mixture of macro-ingredients, such as fat, protein and carbohydrate ingredients plus various minerals, vitamins and other micronutrients as required and then evaporating and spray drying the resultant mixture. A combination of the two processes described above may be used where a base powder is first produced by wet-mixing and spray drying all or some of the macro-ingredients and then dry blending the remaining ingredients, including carbohydrate, minerals and vitamins and other micronutrients, to create a final formula. The reader is referred to Pisecky (1997), Masters (2002) and Vega and Roos (2006) for more details on the fundamentals and practice of spray drying. Liquid formulae are available in a ready-to-feed format or as a concentrated liquid, which requires dilution, normally 1 : 1, with water. The manufacturing processes used for these products are similar to those used in the manufacture of recombined milk. The production of recombined milk has been reviewed extensively in the literature (Zadow, 1982; Kiesecker, 1983; Sjollema, 1987).Effective implementation of current good manufacturing practices is essential to ensure the consistent quality, safety and nutritional adequacy of infant formula products. Facilities and equipment must be of the highest standards. The HACCP (Hazard Analysis Critical Control Point) model is widely used in the industry to prevent contamination. A rigorous quality management system such as the ISO 9000 model

Table 21.9 Regulatory limits for follow-on, second-age, infant formulae manufactured from bovine milk.

Nutrient	Units (per 100 kcal)	Codex Alimentarius*		European Union†	
		Min.	Max.	Min.	Max.
Protein	g	3	5.5	1.8	3.5
Fat	g	3	6	4.0	6.0
Linoleic acid	mg	300	—	300	1200
Linolenic acid	mg	—	—	50	—
Carbohydrate	g	—	—	9	14
Vitamin A	IU	250	750	200	600
Vitamin D	IU	40	120	40	120
Vitamin E	IU	0.7	—	0.75	7.45
Vitamin K	µg	4	—	4.0	25
Vitamin B_1	µg	40	—	60	300
Vitamin B_2	µg	60	—	80	400
Vitamin B_6	µg	45	—	35	175
Vitamin B_{12}	µg	0.15	—	0.1	0.5
Niacin	µg	250	—	300	1500
Folic acid	µg	4	—	10	50
Pantothenic acid	µg	300	—	400	2000
Biotin	µg	0.15	—	1.5	7.5
Vitamin C	mg	8	—	8	—
Choline	mg	—	—	10	30
Inositol	mg	—	—	—	—
Calcium	mg	90	—	50	140
Phosphorus	mg	60	—	25	90
Magnesium	mg	6	—	5.0	15
Iron	mg	1		0.6	2.0
Zinc	mg	0.5	—	0.5	1.5
Manganese	µg	—	—	1	100
Copper	µg	—	—	35	100
Iodine	µg	5	—	10	50
Sodium	mg	20	85	20	60
Potassium	mg	80	—	60	160
Chloride	mg	55	—	50	160
Selenium	µg	—	—	1	9
Nucleotides	mg	—	—	—	5

* Based on data from Codex Alimentarius Commission (1987). Codex standard for follow-on formula, Standard 156-1987, as amended in 1989.

† Reproduced from Commission of the European Communities Directive 2006/141/EC on infant formulae and follow-on formulae. © European Union, http://eur-lex.europa.eu/

is routinely applied to establish and maintain effective processes for all quality-related activities.

The industry is acutely aware that any change to the manufacturing process or formulation may impact the quality of the product. The FDA has classified changes as either major or minor. A major change is considered to be one where the manufacturer's experience or theory would predict possible significant adverse impact on levels of nutrients or availability of nutrients. Examples given of major manufacturing changes include the utilisation of a

new plant or production line, a significant technology change (e.g. from terminal sterilisation to aseptic processing) or a fundamental packaging change (e.g. from metal cans to plastic pouches). Formulation changes such as the addition of a new macronutrient (e.g. fat, carbohydrate or protein), a substantial quantitative change in the protein, fat or carbohydrate content or the addition of new constituents added for their potential nutrient contribution are also considered as major changes. Examples of minor changes include minor reductions or increases in the levels of nutrients, minor changes in time–temperature conditions of preheating during handling of bulk product that cannot reasonably be expected to cause an adverse impact on nutrient levels or nutrient availability, and changes in oxygen content of a packaged product that might have a minimal effect on the level of nutrients (US Food and Drug Administration, 1980).

21.7 PACKAGING OF FORMULAE FOR INFANTS AND YOUNG CHILDREN

Formulae for infants and young children must be stored in tamper-proof packaging that maintains the product quality and integrity throughout a relatively long shelf-life. The traditional packaging format of powder in tinplate cans (400–2000 g) flushed with inert gases such as nitrogen and/or carbon dioxide remains commonplace. In recent times, composite cans, aluminium foil packs and single-serve foil sachets have gained popularity. A recent novel packaging innovation is the packaging of powder in single-serve tablets. A milk-dispensing system that mixes powdered formula, packaged in single-serve portions in sealed capsules, with a precise amount of water into a feeding bottle is a recent innovation. Sterilised ready-to-feed formulae for hospitals and paediatric clinics are typically packaged in single-serve sizes (60–120 mL) in screw-capped glass jars or small plastic bottles.

21.8 FUTURE DEVELOPMENTS

There is still a lot to learn about the compositional, physicochemical and biological aspects of human milk. There will be continuous endeavour by scientists to understand the biologically active components in human milk and the effect of these components on an infant's growth and development. This is turn will aid in the development of new infant formulations that will more closely match human milk and provide optimal growth and development. Research and development will not only focus on the new ingredients and formulations but also on novel technologies, product formats and packaging to ensure new differentiating products can be supplied through cost-effective manufacturing processes. The safety and quality of formulations for infants and young children is likely to remain a primary focus for the formula manufacturers. Labelling laws governing claims and health benefits will continue to challenge the infant formula industry, and it is imperative that manufacturers provide scientifically and clinically substantiated evidence for such claims.

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22
Whey and Whey Products

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22.1 INTRODUCTION

The Code of Federal Regulations defines whey as the liquid substance obtained by separating the coagulum from milk, cream, or skim milk in cheesemaking. Whey obtained from a procedure in which there is insignificant conversion of lactose to lactic acid is known as sweet whey. Sweet whey has a maximum titratable acidity of not more than 0.16%, calculated as lactic acid, and an alkalinity of ash of not more than 225 mL of 0.1 N hydrochloric acid per 100 g. Generally, sweet whey of pH above 5.6 is obtained when rennet is used for milk coagulation. On the other hand, whey with pH less than 5.1 is obtained by coagulating milk with acid and hence is referred to as acid whey (Marella, 2009). Chemically, whey is a mixture of water and different milk components such as fat, protein (mainly water-soluble proteins), lactose, lactic acid, and minerals, predominantly calcium and zinc (Kosikowski, 1979; Parmar, 2003). Whey contains a high concentration of organic matter particularly protein, fat, lactose, and mineral salts, typically 1% protein, 0.5% ash, and 5.0% lactose (Cuartas-Uribe *et al*., 2009). Several of the protein fractions have high nutritional and biological value. Prior to the 1970s, whey was considered a waste product. With the advent of separation and purification technologies, whey was transformed from waste product to an important co-product of cheesemaking (Geoffrey, 2008). In this chapter we briefly present information on different whey and whey protein products, and their biological and nutritional significance. The products and processes described in this chapter represent whey from cows' milk unless otherwise specifically stated.

22.2 SOURCES AND TYPES OF WHEY

22.2.1 Acid and sweet whey

Whey is a co-product of cheesemaking. The majority of whey and whey protein products available in the market are processed from whey when converting milk into cheese or casein. There are two basic types of whey: acid whey and sweet whey or rennet whey. Acid whey is produced from cottage cheese or acid casein manufacture, where milk is coagulated by direct addition of acid as a coagulant. Sweet whey or rennet whey is obtained from the manufacture of cheese products, which involve rennet treatment for milk coagulation (Schmidt *et al*., 1984). Acid whey has high lactic acid and high mineral contents (Table 22.1). The lower pH of acid whey causes insolubility of some individual whey proteins, and hence acid whey is more difficult to process through membrane filtration. Acid whey produced during longer fermentation times of specific cheesemaking processes may also contain high bacterial and enzyme concentrations (Chime *et al*., 2009). Milk protein may be precipitated by the addition of lactic, acetic, or mineral acids such as hydrochloric or sulfuric acid. Whey from coagulating milk protein by lactic acid is called acid whey; it is called industrial whey when agents other than rennet or lactic acid are used for protein precipitation. In general, whey from acid precipitation of milk protein has a pH of 3.9–4.5 and contains less lactose and whey protein than rennet whey (Table 22.1). The ratio of total nitrogen coagulable with heat is higher in acid whey (0.6% of 0.9–1.0% total-N) compared with rennet whey

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Table 22.1. Constituents (%) of rennet and acid whey.

	Rennet whey (Cheddar)	Acid whey (fresh cheese)	Acid whey (casein)
Water	93.3	95.5	94.0–95.0
Dry matter	6.7	6.42	5.4–6.0
Protein	0.6	0.53	0.9
Total N (mg/g)	1.3	1.19	—
Non-protein N (mg/g)	0.34	0.34	—
Protein N (mg/g)	0.95	0.85	—
Soluble N (mg/g)	1.3	1.18	—
Non-protein N (as %N)	26.20	28.6	—
Lactose	5.0	4.40	3.8–4.2
Ash	0.52	0.6	0.7–0.8
Lactic acid	0.14	0.47	≤0.8
Calcium (mg/100 g)	36.50	92.80	—
Zinc (μg/100 g)	11.0	234.00	—

Sources: based on data from Parmar (2003) and reproduced from Sienkiewicz & Riedel (1990), with permission of Th. Mann.

(0.5% of 0.9% total-N). Sulfuric acid whey (also called industrial whey) and lactic acid whey contain higher true protein content (~85 g/kg dry matter) than rennet whey (20 g/kg dry matter). Lactic acid whey such as thermoquarg whey, contains higher amounts of non-protein nitrogen (NPN) than rennet whey or sulfuric acid whey (Sienkiewicz & Riedel, 1990). Acid whey from cottage cheese manufacture is believed to be rich in smaller peptides and free amino acids (Parmar, 2003). Proportionally, production of acid whey and utilization in food applications is significantly smaller than for rennet whey (Sienkiewicz & Riedel, 1990).

22.2.2 Whey from other species

Goat (caprine) and sheep (ovine) milk cheeses and other milk products are commercialized in many countries around the world (Jandal, 1996). There are hundreds of cheese varieties made from goat milk or combinations of goat and other milks. In the USA, about 75% of goats' milk, and almost all (>95%) of the sheep milk produced is used for cheese manufacture (Milani & Wendorff, 2011). Jandal (1996) reported the water-soluble constituents of goats' milk comprised 4.1% lactose, 0.4% whey protein, and 0.8% ash. The α-lactalbumin (ALA) contents in dry whey of Greek goats (Skopelos, Alpine and Saanen breeds) have been reported to be 14.3%, 20.7%, and 22.0%, whereas β-lactoglobulin (BLG) contents of the same breeds were 43.5%, 47.8% and 44.5%, respectively (Moatsou *et al*., 2005). Moreno-Indias *et al*. (2009) analyzed goats' cheese whey collected from farms and cheese factories and reported that whey from farms contained higher dry matter contents (7.1%) than whey from factories (5.1%). Because of inefficient curd cutting and draining, farm whey contained higher fat content, 10% on a dry basis, than factory whey (1.2% fat). Protein contents in farm and factory whey were 14.6% and 18.9%, respectively. Lactoferrin and serum albumin in farm caprine whey were 0.39 mg/mL and 0.60 mg/mL, respectively, whereas factory whey contained 0.15 mg/mL and 0.39 mg/mL of these proteins. Caprine whey samples collected from farms and factories were identical with respect to ALA and BLG content (Moreno-Indias *et al*., 2009). Caprine whey obtained from rennet-coagulated cheddar-type cheesemaking contained 4.0% serum albumin, 9.7% immunoglobulin, 27.0% ALA, and 58.6% BLG on the basis of total protein. In acid whey obtained from goat cheesemaking, serum albumin and ALA were 6.7% and 31.7%, respectively, and other fractions were similar to rennet whey (Casper *et al*., 1998).

Whey proteins account for 17–22% of total proteins in ovine milk. In ovine whey, ALA and BLG are present as major protein fractions whereas immunoglobulin, serum albumin, lactoferrin, casein macropeptides, and peptones due to plasmin-associated breakdown of casein are present in minor quantities (Park *et al*., 2007). Jaeggi *et al*. (2005) reported the total solids of ovine whey to be 9.5%, including 1.8% total protein, 1.5% fat, and lactose and minerals. The proportions of these constituents depend on the seasons and breeds. Jandal (1996) reported that sheep milk contained less lactose (3.7%) but more whey proteins (0.8%) and minerals (0.9%) than goats' milk. A study of Austrian goat and sheep milk also reported a higher percentage of whey protein (0.9%) in sheep milk than in goats' milk (0.5%) (Mayer & Fiechter, 2012). Whey

Table 22.2. Composition of whey from different dairy animals.

Whey components	Buffalo	Goat	Sheep	Yak	Camel	Mare	Cow
Total solid	6.46%[a]	5.08%[c]	9.5%[g]	6.7%[h]	88.5 g/L[l]	—	6.6%[m]
Lactose	4.9%[a]	4.08%[d]	3.7%[g]	4.9%[h]	—	—	5.0%[n]
Fat	0.5%[a]	1.2% of DM[c]	1.46%[g]	0.06%[h]	—	—	0.2%[m]
Mineral/ash	0.35%[a]	0.79%[d]	0.9%[d]	0.6%[h]	—	—	0.2%[m]
Whey protein	0.73%[a]	0.43%[d]	1.75%[d]	1.25%[h]	—	8.3 g/kg[k]	0.8%[m]
α-Lactalbumin	2.45 g/L[b]	19.0% of WP[e1]	13.03% of WP[e2]	0.3%[h]	3.0 g/L[j]	28.55% of WP[k]	24.6% of WP[m]
β-Lactoglobulin	4.04 g/L[b]	45.26 of WP[e1]	62.46% of WP[e2]	0.8%[h]	ND[j]	30.75% of WP[k]	57.9% of WP[m]
Immunoglobulin	—	9.7% of WP[f]	7.39% of WP[f]	0.53% of WP[i]	10.8 g/L[j]	19.77% of WP[k]	4.5% of WP[m]
Serum albumin	0.35 g/L[b]	0.39 mg/mL[c]	4.1% of WP[f]	2.94% of WP[i]	10.8 g/L[j]	4.45% of WP[k]	2.9% of WP[m]
Lactoferrin	—	0.15 mg/mL[c]	—	1.91% of WP[i]	0.7 g/L[j]	9.89% of WP[k]	1.0% of WP[m]
Base whey protein	—	—	—	—	1.1 g/L[j]	—	—

DM, dry matter; WP, whey protein.

Sources: based on data from [a]Macedo *et al*. (1999); [b]Buffoni *et al*. (2011); [c]Moreno-Indias *et al*. (2009): data from whey factory; [d]Jandal (1996); [e1]Moatsou *et al*. (2005): average values of Skopelos, Alpine and Saanen breeds; [e2]Moatsou *et al*. (2005): average values of Frisarta, Chios, and Karagouniko breeds; [f]Casper *et al*. (1998); [g]Jaeggi *et al*. (2005); [h]Neupane *et al*. (1997); [i]Sheng *et al*., 2008; [j]El-Hatmi *et al*. (2007): whey protein contents in colostrum after 6 days of lactation; [k]Malacarne *et al*. (2002), Park *et al*. (2008); [l]Bornaz *et al*. (2009); [m]Blaschek *et al*. (2007): sweet whey from Cheddar cheese; [n]Parmar (2003): sweet whey.

of Greek ovine breeds, namely Frisarta, Chios and Karagouniko, contained 11.7%, 15.2%, and 12.3% ALA and 65.8%, 59.2%, and 62.4% BLG dry basis, respectively. Compared with bovine and ovine whey, caprine whey had more ALA while the BLG percentage was higher in ovine whey (Table 22.2). Casper *et al*. (1998) analyzed ovine sweet whey from Manchego-type cheesemaking and reported 4.1% serum albumin, 7.3% immunoglobulin, 74.0% BLG, and 14.8% ALA on the basis of total whey protein. They also found significant seasonal variations in serum albumin and immunoglobulin concentrations in ovine whey.

In India, Pakistan and Nepal, milk production from water buffaloes exceeds that of cows' milk, and has a substantial share in many other countries, particularly in the Mediterranean region (Pandya & Haenlein, 2009; Food and Agricultural Organization, 2012). Buffalo milk is used to manufacture Mozzarella cheese in Italy, whereas in India most of the cheeses, cheese-like products, and sweets are produced by coagulating buffalo milk. Buffalo milk contains higher percentages of milk constituents than cows' milk (Zicarelli, 2004). Thus buffalo whey is also richer than cow whey in water-soluble constituents such as lactose and minerals (ash) (Spreer & Mixa, 1998). Unlike for cows' milk, there still is a lack of information about protein fractions, especially whey proteins from buffalo milk. Buffoni *et al*. (2011) analyzed whey proteins of Mediterranean water buffalo milk and reported that it contained 4.0, 2.5, and 0.4 g/L of BLG, ALA, and serum albumin, respectively. The molecular sizes of these proteins were 18.3 kDa, 14.2 kDa, and 66.4 kDa, respectively.

Yak milk is used to make special yak cheeses such as Sher, Sewsew, or Chhurpi in the Himalayan regions of Nepal, Bhutan, China, India, Mongolia, and Pakistan (Jianlin *et al*., 2000; Sheng *et al*., 2008). The whey from such sources is primarily used as animal feed. Potassium, sodium, phosphorus, and calcium are 148, 59, 34 and 31 mg/100 g of yak whey, respectively (Neupane *et al*., 1997). Yak cheese whey contains 4.9% lactose and 1.25% total protein including 0.8% BLG, 0.3% ALA, and 0.6% ash. Total solids in yak whey and milk are 6.7% and 19.4%, respectively (Neupane *et al*., 1997).

The major components of camel whey are serum albumin, ALA, immunoglobulins, lactoferrin but no BLG (Table 22.2). Camel whey has been reported to contain greater amounts of defense factors such as lactoferrin and immunoglobulin than bovine whey. The average values of lactoferrin and IgG in raw camel milk were 0.23 and 0.72 mg/mL, respectively. The concentration of these components varied significantly by season; the highest value of lactoferrin was observed in spring, whereas IgG was highest in winter (Konuspayeva *et al*., 2007). Ochirkhuyag *et al*. (1998) characterized whey proteins of Mongolian yak, Khainak, and camel milk. The major components of whey of these species were nearly identical with cow whey. However, there are several reports that colostrum and whey

of camel milk is deficient in BLG (El-Hatmi *et al*., 2007; Laleye *et al*., 2008), which is the major difference between camel and cow whey, where BLG is the most abundant whey protein (Laleye *et al*., 2008).

Mares' milk is seldom used for production of cheese as it is mainly consumed in the form fluid or fermented milk (Marconi & Panfili, 1998). However, there are some reports on water-soluble proteins in mares' milk whey when milk is coagulated. Mares' milk contains both ALA and BLG (Table 22.2), but the latter is less allergenic than that from cow whey. Of the total whey proteins, mares' milk contains 6.6% lysozyme, which is higher than in human milk (1.7%). An antimicrobial feature of mares' milk is mainly due to the presence of lysozyme and to some extent to lactoferrin, which is also present in higher quantity (Malacarne *et al*., 2002).

In the mountains of South American countries, like Peru and Bolivia, llamas are a popular animal for multiple purposes, but not for milk production (Riek & Gerken, 2006).

Reindeer milk is obtained in Nordic countries like Sweden, Finland, Norway, Siberia and dairy products are made (Heikura *et al*., 2006). The milk yield of these animals is relatively low and research information on composition and commercial utilization of milk and whey is lacking.

22.3 WHEY PRODUCTION AND UTILIZATION

Before 1970, the dairy industry used to consider whey as a waste product, and it was drained if not used as animal feed or applied to fields as liquid fertilizer. It was the stringent environmental regulations implemented worldwide that made the cheese industry find ways of utilizing cheese whey for different purposes. Concerted research efforts focused particularly on ultrafiltration technology for whey processing (Geoffrey, 2008). As a result, dairy plants started to process whey into a variety of products such as whey powder, sweet whey, demineralized whey, deproteinized whey, non-hygroscopic demineralized whey, reduced lactose whey, lactose, whey protein concentrate (WPC), and whey protein isolates (WPI). These products have been highly accepted as important ingredients in many foods, feeds, and pharmaceutical and other industrial applications, thereby enhancing the reputation of whey as an important dairy co-product and receiving very good monetary returns, which influenced positively the overall milk income system (Marella, 2009).

Global liquid whey production from cheese and casein manufacturing amounted to 186 million tonnes in 2008, and the average annual growth between the years 2002 and 2008 has been approximately 2% (Affertsholt, 2009). EU countries EU and the USA share 70% of the total world whey production. On the basis of whey type, cheese whey (mostly sweet whey) accounted for 95% of the total whey production, whereas casein whey accounted for 5%. The total global market volume of whey ingredients was 770 000 t in 2008, and from 2005 to 2008 the market increased by 6%. Also, the market for the ingredients with higher additional value such as WPC 80, whey protein hydrolysate (WPH), and WPI is increasing at a much faster rate. The EU and the USA are the largest markets for whey ingredients, sharing 40% and 31% of the global total, respectively. Regarding the utilization of whey ingredients in food consumption, 190 000 t of whey powder, 85 000 t of WPC, and 14 500 t of WPI were used in food consumption in 2008, the major food sectors being dairy, bakery, dry/wet blending, functional food application, infant formula, and confectionary (Affertsholt, 2009).

22.4 MAJOR COMMERCIALIZED WHEY PRODUCTS

Whey is mainly processed by spray drying, membrane filtration technologies, or a combination of both. Depending on the technology used and the whey constituents in the final products, there are different types of processed whey products available on the market. Whole whey products such as condensed whey and whey powders are obtained by concentration of liquid whey, generally using spray-drying technology. Such products proportionally contain all the components of liquid whey except water (Jelen, 1979; Zall, 1984). Fractionated whey products are essentially produced by membrane filtration technology in combination with drying. In fractionated whey products, different protein fractions of whey are separated by membrane filtration and further concentrated by drying (Matthews, 1984). Membrane technology, occasionally in combination with ion exchange, has been frequently used in whey fractionation to produce highly concentrated fractions of WPI. Different types of whey products manufactured through the utilization of membrane technology include WPCs (WPC 34, 50, 60, 75, and 80 depending on the protein content), WPI, demineralized whey, reduced lactose whey, deproteinized whey, etc. (Marella, 2009). Lactose and milk minerals are important byproducts of whey filtration.

22.4.1 Whey powder

Depending on the type of whey used, whey powder can be categorized as sweet whey powder or acid whey powder. Liquid whey is processed through a series of unit operations such as clarification, pasteurization, vacuum evaporation, spray drying, and crystallization. The selection and variation of these operations depends on the stability and

functionality of the products (Marella, 2009). Whey powder is a complex mixture of lactose, proteins, and minerals, with a minimum amount of moisture and fat. The constituents of whey powder vary in quantity and in physical state and association. Lactose is the largest structural element of whey powder particles and is available in amorphous or crystalline form. Fat (globular and non-globular), protein, and air are dispersed in a continuous phase of amorphous lactose. Variation in processing conditions and the resulting physical state of lactose governs the hygroscopic or non-hygroscopic nature of whey powder (Banavara *et al*., 2003). Different constituents of whey powder are proteins (11–14.5%), fat (0.5–1.5%), and ash (8.2–12.3%). Total solids content in whey powder ranges from 95 to 96.5% (Marella, 2009). There is compositional difference between acid and sweet whey powders in terms of protein, acidity, and mineral elements. Calcium and phosphorus in acid whey powders (1.8% and 1.1%, respectively) are relatively higher than in sweet whey powders (0.6% and 0.8%, respectively). Acid whey powders are also richer in zinc content (41.7 ppm) compared with sweet whey powders, which contain only 9.0 ppm. Sodium, potassium, most phospholipids, and vitamins (riboflavin, thiamine, and ascorbic acid) contents are similar in acid and sweet whey powders (Mavropoulou & Kosikowski, 1973).

The Code of Federal Regulations has defined dried whey as "the dry substance obtained by the removal of water from whey, while leaving all other constituents in the same relative proportions as in whey." The ingredients should contain protein (10–15%), fat (0.2–2.0%), ash (7–14%), lactose (61–75%), and moisture (1–8%). Solids content and titratable acidity may be variable. The limits of heavy metals and other impurities are also included in the standard document. Dry (dried) whey sold to food manufacturers should be labeled "dry (dried) (sweet or acid) whey" or "dry (dried) whey, (% titratable acidity)". Whey, concentrated whey, or dry (dried) whey in a finished food product should be listed as "whey" (Department of Health and Human Services, 2011). The American Dairy Product Institute has prescribed the specifications of extra grade dry whey as milk fat 1.5%, moisture 5.0%, scorched particles 15.0 mg, standard plate counts 30 000/g, coliforms 10/g. It should be a free-flowing powder with uniform color and free from undesirable flavors (ADPI, 2002). Production of whey powder by membrane technology is accomplished through microfiltration followed by nanofiltration or reverse osmosis. The retentate from nanofiltration or reverse osmosis is spray dried after evaporation to produce whey powder. A dilute solution of water-soluble mineral salts is lost in the form of permeate (Fig. 22.1).

22.4.2 Whey protein concentrates

WPCs are white to light cream colored containing 34–80% protein. Introduction of ultrafiltration technology in the dairy industry has successfully made the separation of whey protein so that they can be concentrated as important value-added products. WPC with variable protein contents can be manufactured by sequential steps involving pretreatment, ultrafiltration/diafiltration, evaporation under vacuum, and spray drying (Abd El-Salam *et al*., 2009). Ultrafiltration is used as an essential step of WPC manufacture, either to manipulate the protein content and/or to reduce other components such as fat, lactose, and mineral contents. Whey, if processed without any pretreatments, will produce WPCs with all the components present in the liquid whey (Marella, 2009). Depending on the protein content, WPC is designated as WPC 34 (34–36%), WPC 50 (50–52%), WPC 60 (60–62%), WPC 75 (75–78%), and WPC 80 (80–82%). The two concentrates that are commonly produced and widely used are WPC 34 and WPC 80. WPC 34 contains 34–36% protein, 48–52% lactose, 3.0–4.5% fat, 2.5–8.5% ash, and 3.0–4.5% moisture. In WPC 80, the proportion of lactose is reduced to 4.0–8.0%, whereas the proportion of protein is increased to 80–82%. WPC 80 contains a higher percentage of fat (4.0–8.0%) than WPC 34, but the proportion of ash and moisture is similar in both types. Of the total protein in WPCs, the major protein fraction is BLG (57–70%), followed by ALA (15–25%), immunoglobulin (12–19%), and bovine serum albumin (BSA, 4–6%). Calcium, potassium, and phosphorus are the major minerals, found at 0.75%, 0.45%, and 0.2%, respectively (Abd El-Salam *et al*., 2009). High protein concentration in WPC 80 is achieved by filtration of pasteurized clear whey through a two-stage ultrafiltration system. The first stage produces a retentate with approximately 15–20% solids, which is further passed through diafiltration in combination with ultrafiltration (Fig. 22.1). The final retentate is evaporated before spray drying. Alternatively, a nanofiltration system can be used instead of evaporation, prior to spray drying. Incorporation of a third ultrafiltration step has also been utilized for production of low fat WPC 80 (Marella, 2009). A wide-pore ultrafiltration system has been described for the manufacture of ALA-enriched WPC 80 (Fig. 22.2) (Marella *et al*., 2011).

22.4.3 Whey protein isolate

WPI is the highest protein-containing whey protein product, with more than 90% water-soluble milk protein. Similar to WPC 80, WPI is also a white to light cream colored powder with a bland taste and clean flavor (Marella, 2009). WPI is manufactured by removing sufficient

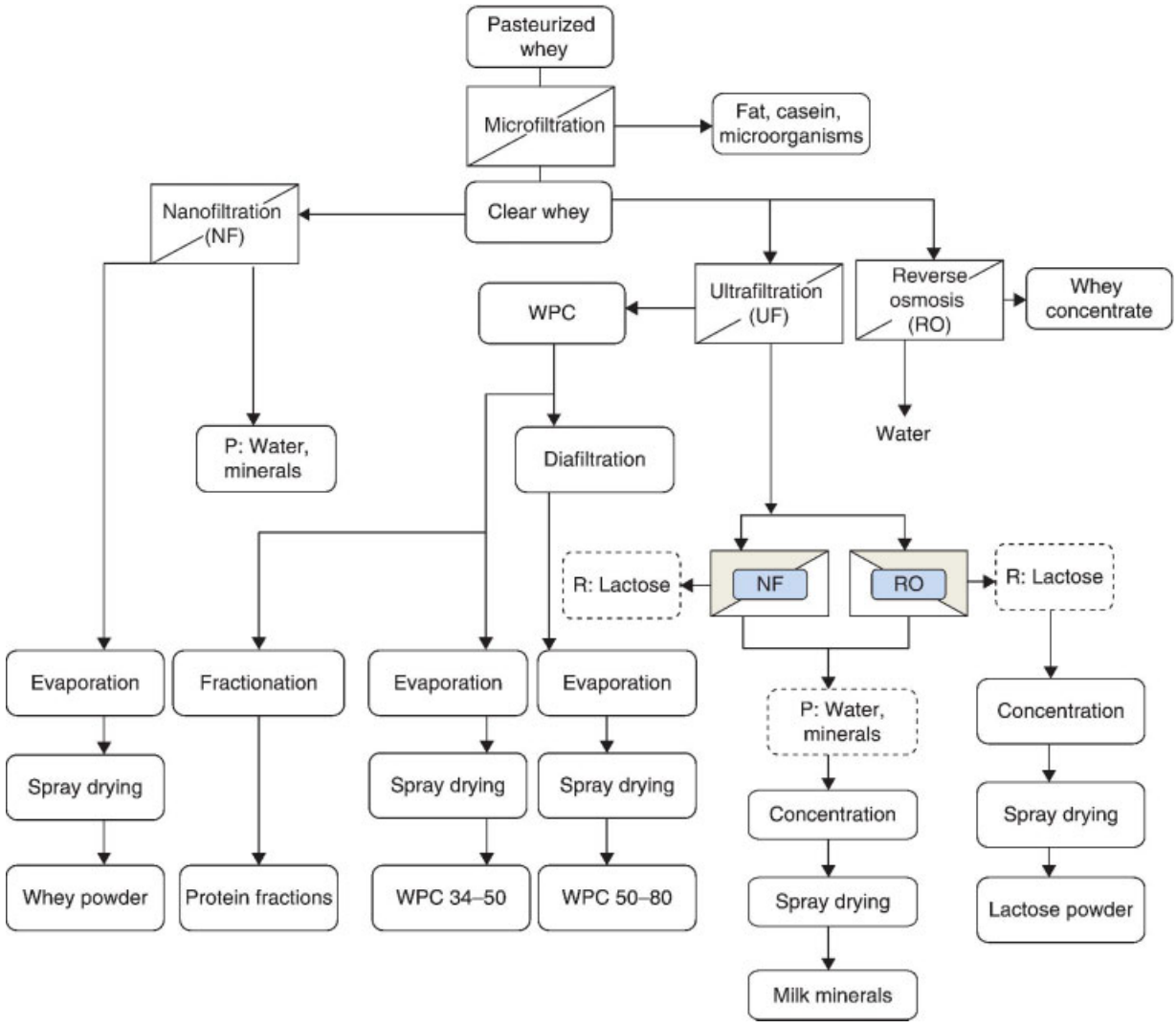


Figure 22.1. Whey powder and whey protein concentrates manufacturing flow chart. R, retentate; P, permeate. Based on data from Ji & Haque (2003), Rektor & Vatai (2004), Arzu *et al.* (2009), Outinen *et al.* (2010) and USDEC (2012). Data courtesy of USDEC. Reproduced from Reference manual for U.S. whey and lactose products (www.usdec.org).

non-protein constituents from whey, so that the finished dry product contains not less than 90% protein (USDEC, 2012). WPI is commercially manufactured by membrane filtration or ion exchange chromatography. In the ion exchange process, protein from whey is separated by adsorption with ion exchange resin (Foegeding & Luck, 2002). The adsorbed protein is recovered by washing the resin, further concentrated by the ultrafiltration process, and finally spray dried into powder. The other method involves three-stage alternate ultrafiltration and microfiltration systems. In this process, whey is ultrafiltered and the retentate is fed to subsequent microfiltration. The permeate of microfiltration is ultrafiltered again. The final retentate may be directly spray dried or further concentrated using nanofiltration before spray drying (Marella, 2009). According to USDEC (2012), a typical WPI contains 90–92% protein, 0.5–1.0% lactose, 0.5–1.0% fat, 2–3% ash, and 4.5% moisture. It has been reported that BLG is a major protein fraction of WPI accounting for 32–62%, followed by glycomacropeptide (GMP) (17.2–19.7%). It contains relatively less ALA (4.2–18.4%) and BSA (0.4–8.1%) than WPC 80 (Abd El-Salam *et al.*, 2009).

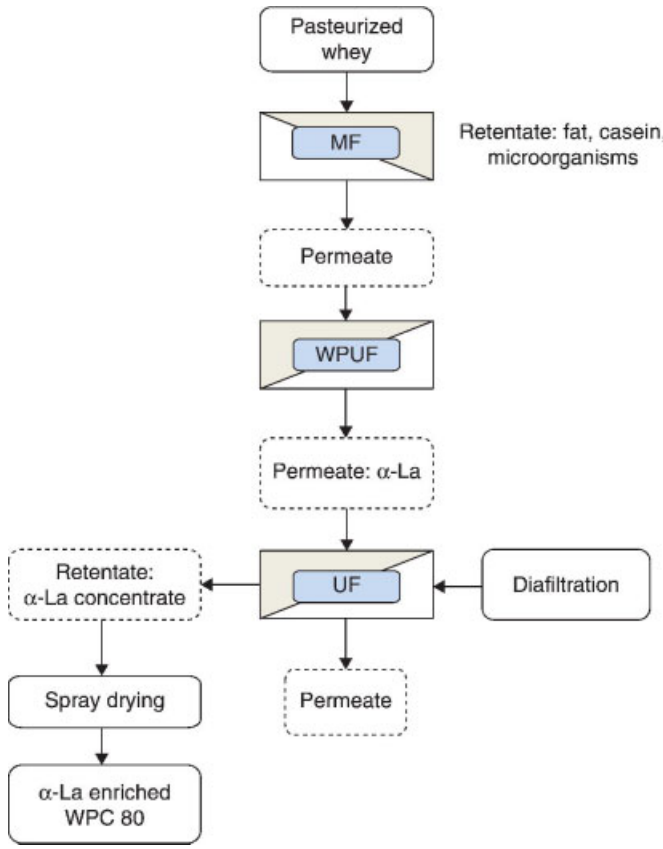


Figure 22.2. Manufacturing flow chart of α-lactalbumin-enriched WPC 80. α-La, α-lactalbumin; MF, microfiltration; UF, ultrafiltration; WPUF, wide pore ultrafiltration. Reproduced from Marella *et al.* (2011), with permission of Elsevier.

The WPI produced by the ion exchange process generally has low concentrations of immunoglobulins, lactoferrin, lactoperoxidase, and GMP, while the WPI made by membrane processing will have different levels of these fractions, depending on the number of microfiltration steps involved (Marella, 2009).

22.4.4 Whey protein fractions

The major protein fractions found in whey are ALA, BLG, BSA, and immunoglobulins. Most of the nutritional, functional, physiological, and therapeutic properties of whey proteins are due to these protein fractions. ALA is rich in tryptophan, which is an essential amino acid. In its native form, ALA contains 1 mole of bound calcium per mole of protein, which stabilizes the tertiary structure of the protein. BLG is more popular because of its functional properties, such as foaming and gelation. Lactoferrin and lactoperoxidase are minor but commercially important protein fractions found in whey protein (Bramaud *et al*., 1997; Etzel, 2004). In addition to these, sweet type whey also contains GMP. The individual protein fractions differ in their physical and chemical properties such as molecular weight, molecular size, and isoelectric pH (Marella, 2009).

22.4.4.1 α-Lactalbumin

The major water-soluble protein fractions of milk are ALA and BLG. These fractions together account for 3.0–5.1 g/L of milk and more than 70% of the whey protein. BLG accounts for almost 50% of the total whey protein, whereas ALA accounts for 20% (Abd El-Salam *et al*., 2009; Marella *et al*., 2011; USDEC, 2012).

ALA is a small globular protein with molecular mass of 14.2 kDa (Fox & McSweeney, 2003). It is rich in tryptophan (5.3%), an essential amino acid, and sulfur-containing amino acids such as cysteine (5.8%) and methionine (0.9%). It is also a rich source of

branched-chain amino acids, containing 10.4% leucine, 6.4% isoleucine, and 4.2% valine. In ALA, essential amino acids constitute 47.2% of total weight, whereas branched-chain amino acids (leucine, isoleucine, and valine) share 21% of its weight (Etzel, 2004). This is a calcium-binding protein and has a high affinity for other divalent and trivalent metal ions, including zinc, manganese, cadmium, copper, and aluminum (Heine *et al*., 1991). The biological function of ALA is of great significance as it is the regulatory component of lactose synthesis in mammals. It strongly facilitates glucose binding to UDP-galactose β-*N*-acetylglucosaminide β(1→4)-galactosyltransferase 1, thereby catalyzing the transfer of galactose to glucose during lactation (Greene *et al*., 1999). ALA accounts for 30% of total protein in human milk and there is almost a 72% similarity between human and bovine ALA. A purified bovine ALA is thus widely used to manufacture infant milk formulations (Marella *et al*., 2011; USDEC, 2012). ALA is also used as a nutrient in sports drinks, as it is a good source of branched-chain amino acids. Reports suggest that administration of branched-chain amino acids, particularly leucine, has an anabolic effect on protein metabolism. Such effect may either be due to an increase in the rate of protein synthesis or by a decrease in the rate of protein catabolism or both (Blomstrand *et al*., 2006).

22.4.4.2 β-Lactoglobulin

BLG is the other major whey protein in milk of ruminant species. It is present in many, but not all, mammalian species. It is not present in the milk of rodents, human, camel, and lagomorph species. BLG accounts for the highest proportion (50–55%) of total whey proteins, and for about 10% of total milk proteins (Kontopidis *et al*., 2004). It is a small, water-soluble, acid-stable protein molecule which exists as a monomer with a molecular mass of 18 kDa at pH below 3, whereas at normal whey pH (5–7) BLG exists as a dimer with a molecular mass of 36 kDa (Uhrínová *et al*., 2000). It is an excellent source of essential and branched-chain amino acids, which account for 48% and 25% by weight of total BLG, respectively. Sulfur-containing amino acids, namely cysteine and methionine, account for 2.8% and 2.9% of its weight, respectively (Etzel, 2004). At 72°C, BLG is denatured into gel, and this functional property has been exploited in many food applications such as fish products, formulated foods, acidic protein fortified beverages, and meat processing (Marella, 2009). Trichloroacetic acid precipitation, thermal precipitation, peptic hydrolysis, and precipitation using the salting-out process can be applied for the selective isolation of BLG. The isolated BLG is ultrafiltrated and spray dried in to BLG powder (Konrad *et al*., 2000).

22.4.4.3 Glycomacropeptide

GMP is a 64-amino-acid glycophosphopeptide, abundantly produced during cheesemaking. During rennet action, bovine κ-casein is broken down by chymosin into *para*-κ-casein and water-soluble GMP. *Para*-κ-casein remains in the curd but GMP ends up in the whey. GMP constitutes 15–20% of the protein in bovine cheese whey, and is currently sold as a food ingredient (Brody, 2000; Ney *et al*., 2009; Choi *et al*., 2012). GMP has a unique profile of essential amino acids compared with other dietary proteins. While pure GMP is free of phenylalanine, GMP isolated from cheese whey may be contaminated with other whey proteins and small amounts of phenylalanine (Ney *et al*., 2009). According to Etzel (2004), essential amino acids and branched-chain amino acids comprise 47% and 22.5%, respectively, of the total GMP weight.

GMP is a low molecular mass protein of 8.0 kDa which remains negatively charged even at pH 3. Stability of GMP at low pH does not allow it to be separated even by cation exchange or by polyacrylamide gel electrophoresis. Isolation and quantification procedures for GMP require the use of precipitants, which precipitate all the whey proteins leaving only the GMP in solution (Brody, 2000). GMP has been purified from sweet whey by different techniques, including precipitation by heat, ethanol or trichloracetic acid, followed by separation using ultrafiltration, gel chromatography, hydrophobic interaction chromatography, and ion exchange chromatography (Bonnaillie & Tomasula, 2009). Owing to its low isoelectric pH, GMP with higher yield and purity can be produced by using anion exchange chromatography (Li *et al*., 2010). Commercially available GMP is a light colored, homogeneous, free-flowing powder with a clean and bland flavor. The product is typically pure (>97% purity) and contains less than 1.0% lactose, 0.6% fat, 6.3% ash, and 94% total solids (USDEC, 2012).

22.4.4.4 Bovine serum albumin

BSA is a globular protein with pH 5.1 and molecular mass of 66 kDa. It accounts for about 5–10% of total whey proteins, and consists of 582 amino acid residues with one free sulfhydryl group and 17 intramolecular disulfide bonds (Damodaran & Paraf, 1997; Etzel, 2004; Marella, 2009). Milk contains 0.4 g/L of BSA. It also has the ability to bind fatty acids with their amino acids (Yalçın, 2006; Marella, 2009).

22.4.4.5 Lactoferrin

Lactoferrin is an iron-binding glycoprotein that is part of the transferrin protein family along with serum transferrin, ovotransferrin, and melanotransferrin (González-Chávez

et al., 2009). The lactoferrin molecule has a molecular mass of 80 kDa, and comprises 700 amino acid residues (Ward *et al*., 2005; González-Chávez *et al*., 2009; Marella, 2009). Structurally, the lactoferrin is folded into two globular units, with each unit being able to bind 1.4 mg of iron per gram of protein. Bovine lactoferrin is somewhat similar in structure to the human form, having approximately 70% similarity in amino acid composition (Sharma & Shah, 2010; Manzoni *et al*., 2011). Lactoferrin is produced by mucosal epithelial cells in various mammalian species, including humans and bovine. Lactoferrin content in milk varies depending on the species. Cows' milk contains 100–400 mg/L, which is much lower than human milk, which contains 1–3 g/L. However, advances in separation techniques have made it possible to produce large amounts of bovine lactoferrin with high purity (Wakabayashi *et al*., 2006). Sweet whey contains 30–100 mg/L of lactoferrin, which is commercially separated and concentrated using cation exchange cross-flow membranes. Commercially, 90% pure lactoferrin is available in the form of powder that contains less than 5% moisture and more than 90% protein (USDEC, 2012).

22.4.4.6 Lactoperoxidase

Lactoperoxidase is a heme-containing glycoprotein with a single chain of 612 amino acid residues that has a molecular mass of 78 kDa (Sisecioglu *et al*., 2009). It is a native milk enzyme having antimicrobial properties and is present in whey at a concentration of 1–30 mg/L. Whey-based lactoperoxidase is relatively heat stable (Marella, 2009; USDEC, 2012). In the presence of hydrogen peroxide, lactoperoxidase catalyzes the oxidation of halides and pseudohalides to generate products with wide antimicrobial activity (Sisecioglu *et al*., 2009). Lactoperoxidase, thiocyanate, and hydrogen peroxide constitute one of the important milk defense systems for restricting the growth of microorganisms. Lactoperoxidase catalyzes the oxidation of native thiocyanate (SCN^-) with hydrogen peroxide (H_2O_2), resulting in the production of antibacterial hypothiocyanate ($OSCN^-$). This oxidation product causes lysis of membranes (Çankaya *et al*., 2010). On a commercial scale, lactoperoxidase is manufactured from whey by companies such as Glanbia Nutritionals, USA, and Armor Proteins, France (Marella, 2009).

22.4.4.7 Immunoglobulins

Immunoglobulins are a complex group of proteins comprising IgG, IgA, and IgM. The immunoglobulins are present in milk at a concentration of 0.6–1.0 g/L, and constitute about 2% of total milk proteins (USDEC, 2012) and 10–15% of total whey proteins. Immunoglobulins are glycoproteins and are either monomers or polymers, made up of two light chains (~20 kDa) and two heavy chains (~50–70 kDa) linked together by disulfide bonds (Madureira *et al*., 2007; Marella, 2009). Bovine whey and colostrum include IgA and secretory IgA, IgG1, IgG2, and IgG fragments, IgM, IgE, J-chain or components, and free secretory components. However, up to 80% (w/w) of all immunoglobulins in milk or whey is accounted for by IgG (Madureira *et al*., 2007). These fractions are produced on a commercial scale by companies such as Glanbia Nutritionals, USA, and Carbery, Ireland (Marella, 2009).

22.4.5 Non-protein whey products

22.4.5.1 Lactose

Lactose is a disaccharide with the empirical formula $C_{12}H_{22}O_{11}$ that is hydrolyzed by the enzyme β-galactosidase into glucose and galactose molecules. It has a molecular mass of 342.30 kDa (USDEC, 2012). Lactose is a reducing sugar and reacts with the amino groups of proteins. The reaction is called the Maillard reaction, which causes significant loss of nutritive value, as well as brown discoloration of the products and flavor change. Lactose content in cow milk is approximately 4.6%, and 74–75% in sweet whey powder, whereas in WPI it is less than 1% (Early, 1992; USDEC, 2012). Implementation of membrane technology in whey processing has created an opportunity for the production of commercial lactose as an important by-product. Several crystalline forms of lactose with differing physical properties are available in the market. A non-hygroscopic α-lactose monohydrate is the most predominant commercial form, which is produced on crystallization at a temperature below 93.5°C (Yang & Silva, 1995). Lactose is used in sweets, confectionery, bakery, and sausages. It provides good texture, and is also used as a color and water binder (Vesa *et al*., 2000).

22.4.5.2 Milk minerals

The mineral content in milk is relatively small (about 8–9 g/L), which contains both cations in the form of calcium, magnesium, sodium, and potassium, and anions in the form of inorganic phosphate, citrate, and chloride (Gaucheron, 2005). The mineral content of milk varies due to a number of factors such as stage of lactation, nutritional status of the animal, and environmental and genetic factors (Kevin, 2006). Milk mineral is the generic name given to the dried precipitate obtained from microfiltration of whey (USDEC, 2012). Milk mineral is an important value addition to the permeate, which is produced as a result of membrane separation of whey during manufacture of different whey protein fractions. Milk mineral contains calcium and phosphorous in the form of calcium phosphate. Calcium phosphate in the permeate is precipitated under controlled conditions, and

the precipitate is washed and dried into powder that contains 30% calcium (Harju, 2001). To recover water-soluble salts from whey, whey permeate is passed through nanofiltration. The permeate of this nanofiltration is filtered through reverse osmosis and dried after evaporation. This gives whey salt powder that is rich in water-soluble minerals such as potassium and sodium (Harju, 2001). Milk mineral has several usages. Typically, it can be used as nutritional supplement in different foods and calcium fortification in foods, juices, and drinks (USDEC, 2012).

22.4.6 Products from non-bovine whey

22.4.6.1 Whey cheeses

Whey cheeses are manufactured all over the world by traditional methods on a small scale or by more standardized industrial processes. They have distinct names on the basis of the region or countries (Pintado *et al*., 2001). There are many whey cheeses that were originally produced from caprine or ovine whey as by-products. However, over the course of time, their consumption increased and people started to produce them from cows' whey or even from milk as a major product. Ricotta, Manouri, and Myzithra are examples of some commercially produced whey-based cheeses (Díaz *et al*., 2004; Ribeiro & Ribeiro, 2010). Manouri is a traditional Greek cheese made from whey obtained during the production of caprine and/or ovine milk cheese. Although it is a whey cheese, nowadays Manouri is also produced as a major dairy product from ovine milk (Lioliou *et al*., 2001). Myzithra is another popular whey cheese in Greece, which is produced from the whey of Feta and other harder cheeses. Myzithra contains 75% moisture, 1–1.5% salt, 15–20% lipids, and has a relatively high pH of 6.8 (Dermiki *et al*., 2008). Ricotta is a variety of cheese that originated in Italy, and is prepared from a blend of rennet whey and whole milk. Normally, the proportion of whey to whole milk is kept to 80 : 20 at the start of Ricotta manufacture. The composition and moisture content of Ricotta cheese depends on several factors, such as the recipe for whey and whole milk, methods of manufacture, and the source of whey (Modler, 1988). Ricotta cheese is also manufactured using ultrafiltration of whey, with reduced cost and improved efficiency (Pintado *et al*., 2001). Gjetost, Broccio, Requeson, and Requeijao are other cheese types that are produced from whey of bovine, caprine, and/or ovine milk (Díaz *et al*., 2004; Dermiki *et al*., 2008; Ribeiro & Ribeiro, 2010).

22.4.6.2 Other whey products

Commercialized products from non-bovine whey are rare in the market. There is limited information describing such products in detail. A commercial formula of ovine whey powder, "Alim15," manufactured by Alimenta s.r.l., Macomer, Italy, with a composition of 70% lactose, 15% proteins, 2% fat, 4% water, 9% ash, and particle size of under 150 μm has been reported. When whey powder was incorporated into Italian cookies (Amaretti), the cookies resulted in more homogeneous internal structure and increased shelf-life without affecting the sensory properties (Secchi *et al*., 2011). Another report concerns Mt. Capra, which has been farming herds of goats and producing concentrated goat mineral whey in an appropriately dehydrated powder form since 1928. The Capra mineral whey produced by concentrating 2 quarts (1.9 L) of liquid whey to produce 1 tablespoon (15 g) of powder is considered a nutrient-packed concentrate. The mineral whey powder has been used as a nutritional supplement and has therapeutic benefit for patients with arthritis, allergies, asthma, digestive distress, bowel toxicity, muscular aches, and pains. It also supports replacement of electrolytes, supplies trace minerals, and allows the normal intestinal flora of an individual to flourish (Wellman, 2005).

There are some studies that suggest the possibility of exploiting membrane filtration technologies for the manufacture of whey products from caprine and ovine wheys. Caprine and ovine WPCs with 65% protein were manufactured in a laboratory by ultrafiltration of cheese whey followed by diafiltration of retentate. WPCs were reported to have functional and nutritive qualities comparable, and in some cases superior, to bovine WPC (Casper *et al*., 1999). Another study reported the laboratory-scale production of caprine and ovine WPCs by lyophilization of filtered fat-free whey followed by reconstitution to obtain threefold total solids compared with whey, and dialysis at a molecular mass cut-off of 10 kDa. Lactose content in ovine WPC was lower than that in caprine WPC, but the protein content was higher in ovine WPC. Sodium, calcium, and potassium contents in ovine and caprine WPC were almost similar but less than that in bovine WPC. In both ovine and caprine WPC, BLG was the highest occurring whey protein, followed by ALA. In caprine WPC, IgG was higher in concentration than serum albumin, whereas in ovine WPC serum albumin was found to be higher (Pintado *et al*., 1999). The production of liquid whey protein concentrate (LWPC) has been described by batch ultrafiltration of whey using an organic membrane of 5.5 m^2 installed area and 20 kDa molecular cut-off. The retentate was heated at 90°C for 1 min to precipitate denatured whey proteins and homogenized at 100 bar to regulate the particle size at 10 μm. Utilization of LWPC as a replacement for skim milk powder in set yogurt manufacture resulted in increased protein and total solids content (Henriques *et al*., 2011). Pereira *et al*. (2002) have described value addition of ovine

cheese whey and deproteinized whey by thermocalcic precipitation and microfiltration followed by diafiltration-aided ultrafiltration. Compared with conventional ultrafiltration or microfiltration, ultrafiltered–diafiltered retentate powders showed the highest protein and lowest calcium concentration. Hernández-Ledesma *et al*. (2002) isolated ovine and caprine BLG isolate with 92% purity from sweet and acid whey by the trichloroacetic acid precipitation method. The isolate was hydrolyzed with trypsin, chymotrypsin, proteinase K, and thermolysin to produce WPHs. Hydrolysates produced with enzymes of microbial origin demonstrated higher angiotensin-converting enzyme (ACE) inhibitory activity.

Direct incorporation of liquid whey into food and beverages has also been studied to find ways of utilizing whey, as processing small amounts of whey into products is barely feasible. Goat cheese liquid whey of 0.4% fat and 0.8% acidity was vacuum filtered and 10% (w/v) refined sugar and 0.2% stabilizer was added. It was pasteurized at 75–80°C for 15 min and cooled to 20°C followed by addition of 7.0% fruit (strawberry or peach) pulp. The beverages thus produced exhibited commercialization possibilities as an alternative use for unused goat cheese whey (Tranjan *et al*., 2009). Similarly, goat milk liquid whey was used to replace ice in goat meat (chevon) nugget formulations. No differences in most physicochemical, textural, and sensory attributes of the nuggets were observed. Addition of nuggets in place of ice increased emulsion stability, ultimately increasing the yield of the final product. Even 100% replacement of ice by liquid goat whey did not result in any adverse sensory properties of the product (Das & Sharma, 2009). In an attempt to increase the positive use of whey, a study has described the successful incorporation of buffalo whey in preparation of fermented beverages in Brazil. A substrate with buffalo cheese whey 35%, soymilk 30%, and cow milk 35% was fermented by a mixed culture of *Lactobacillus casei* Shirota and *Bifidobacterium adolescentis* at 37°C for 8 hours. A vanilla-flavored fermented beverage was evaluated for chemical, microbiological, and sensory characteristics during storage periods of 28 days at 4°C. The beverage did not show any chemical, microbiological, or sensory defects during the storage period (Macedo *et al*., 1999).

Even though there are few non-bovine whey products available on the market, the reviewed information indicates the possibilities of utilizing the whey-processing techniques used in cow whey processing to process whey from non-bovine animals. Furthermore, there are ways of utilizing liquid whey in foods and beverages so as to benefit from the highly nutritional whey components and reduce whey loss in the environment as a pollutant.

22.5 NUTRITIONAL VALUE OF WHEY COMPONENTS

Historically, whey had been considered a problem for cheese manufacturers. They used to dispose whey by spraying on fields, discharging in water bodies, draining in sewers or giving away as animal feed for almost no return. During the 1970s, discarding whey in the environment was banned (Geoffrey, 2008). This created opportunities for better management of whey, which ultimately led to the development of modern whey-processing techniques, although the production of popular whey cheeses such as Ricotta, Manouri, Mizithra, Gjetost, Ziger, and Mascarpone in countries like Italy, Greece, Norway, and Switzerland, among others, has a long tradition. Nowadays, whey is considered as an important co-product of the cheese industry, because several value-added ingredients are produced from it. Such whey-based ingredients are rich in protein, including bioactive peptides, sugar, and minerals (Choi *et al*., 2012). Manipulation of membrane technology has been fully exploited to produce the ingredients with enhanced concentration of specific single whey fractions. There are several whey-based ingredients with high nutritional, biological, and functional characteristics, and these ingredients are used in foods, feeds, pharmaceuticals, and specialized nutrition (Bramaud *et al*., 1997).

Whey contains most of the water-soluble components of milk except casein-bound minerals. It contains all the water-soluble milk protein fragments such as lactoglobulin and lactalbumin, serum albumin, lactoferrin, lactoperoxidase, GMPs, and immunoglobulins that are present in milk (Etzel, 2004). Whey also contains milk sugar (lactose), water-soluble milk vitamins, and minerals. Quantitative occurrence of these components, resistance to processing applications, and their physiological functions and bioavailability to the consumer are important factors to consider while discussing the nutritional significance of whey-based ingredients (Forsum & Hambraeus, 1977; Solak & Nihat, 2012).

22.5.1 Protein and bioactive peptides

22.5.1.1 Whey protein quality

Nutritional comparison of different proteins is generally carried out on the basis of three parameters: biological value, protein efficiency ratio, and net protein utilization. The biological value is the amount of body protein in grams of an adult that can be replaced by 100 g of protein in the diet. The biological value of whole egg protein is considered to be 100. The biological value provides the amount of diet protein that can be efficiently utilized by the body (Renner, 1983). The protein efficiency ratio is the gain in weight of growing subjects produced by 1 g of

Table 22.3. Nutritional properties of some proteins of animal origin.

Protein types	Biological value	Protein efficiency ratio	Net protein utilization
Milk	91	2.5	82
Casein	77	2.5	76
Whey protein	104	3.2	92
Egg	100	3.9	94
Beef	80	2.9	73

Sources: based on data from Hoffman & Falvo (2004), Sarwar (1997) and USDEC (2012). Data courtesy of USDEC. Reproduced from Reference manual for U.S. whey and lactose products (www.usdec.org).

dietary protein. The protein efficiency ratio value of casein is 2.7 and is used to compare other proteins. The net protein utilization and biological value are similar, except that the net protein utilization is measured using nitrogen ingested instead of nitrogen absorbed, as in the biological value (Hoffman & Falvo, 2004). The nutritional properties of different proteins of animal origin are compared in Table 22.3. It is clear that whey proteins are superior to gross milk proteins and casein on the basis of biological value, the protein efficiency ratio, and net protein utilization, and are similar to egg protein.

Whey proteins contain high proportions of essential and sulfur-containing amino acids. They are considered to be superior to casein on the basis of essential and sulfur-containing amino acid content. Total whey proteins contain 609 mg/g essential amino acids, whereas casein contains 511 mg/g. Sulfur-containing amino acid content in whey proteins and casein is 52 and 32 mg/g, respectively (USDEC, 2012). With a high concentration of sulfur-containing amino acids, immune function is reported to be enhanced through intracellular conversion to glutathione (Marshall, 2004).

22.5.1.2 Whey protein digestion and absorption

Whey proteins are also called "fast proteins." They are not coagulated by acid in the stomach, and rapidly reach the intestine, where they remain for a long time for a sustained absorption. They are absorbed slowly and completely in the small intestine (Marshall, 2004). The structure of lactoferrin is abnormally resistant to proteolytic enzymes. Extreme stability is the reason that lactoferrin passes through the gastrointestinal tract. This can be monitored, for substantial quantities of lactoferrin are found in the stool of subjects administered lactoferrin (Lonnerdal, 2009). Lactoferrin also improves iron intake in human subjects. Reports suggest that iron status (hematocrit) of infants fed formula with bovine lactoferrin was better than that of infants fed formula with the same amount of iron from other sources but no lactoferrin. In a study conducted with female long-distance runners, subjects given iron only showed significantly lower serum iron, ferritin and red blood cell counts after the intervention, whereas the iron level was maintained in the group given lactoferrin. (Lonnerdal, 2009)

22.5.1.3 Biological functions of whey proteins

Biological functions of whey proteins are associated with the defined functional roles of specific peptides. Immunoglobulins are known for their immunity-enhancing and disease-prevention properties and are used in nutritional formulae (Yalçın, 2006). Lactoperoxidase is known to have preservative action. The lactoperoxidase enzyme system catalyzes peroxidation of thiocyanate, producing halides (such as iodine and bromium), which ultimately generates antibacterial products (Marshall, 2004). ALA regulates lactose synthesis in mammals by facilitating the transfer of galactose to glucose during lactation (Greene *et al*., 1999).

With respect to biological values, BLG is of value, as are other fractions of whey protein. The globular structure of BLG remains stable against the acids and proteolytic enzymes present in the stomach. Such stability of BLG is related to its function as a resistant carrier of retinol (a provitamin A) from the cow to the young calf. However, this function appears to be less important for human babies, which may explain why BLG is absent in human milk (de Wit, 1998). BLG is a rich source of cysteine, an essential amino acid that stimulates glutathione synthesis by the liver for protection against intestinal tumors (McIntosh *et al*., 1995). Whey proteins have the ability to bind minerals. ALA and BLG can bind calcium, copper, iron, manganese, magnesium, phosphorus, and zinc, and can function as carrier of these minerals (Morris & Fitzgerald, 2009). BSA is responsible for transportation of insoluble free fatty acids (de Wit, 1998; Tunick, 2009).

22.5.1.4 Antimicrobial activity of whey proteins

Lactoferrin's ability to respond to a variety of physiological and environmental changes is a reason to consider it one of the key components in the host's first line of defense

(Valenti & Antonini, 2005). It has strong antimicrobial activity against a wide spectrum of bacteria, fungi, yeasts, viruses, and parasites (Morris & Fitzgerald, 2009). It is also known to have anti-inflammatory and anticarcinogenic activities, as well as several enzymatic functions (González-Chávez *et al*., 2009). Jenssen and Hancock (2009) have reviewed several antimicrobial functions of lactoferrin, and reported that it has inhibitory activity towards Gram-positive and Gram-negative bacteria, enveloped and naked viruses, yeasts and fungi, as well as parasites and other eukaryotic microbes. Lactoferrin is also effective against enteric pathogens; it causes growth inhibition by impairing the function of surface expressed virulence factors thereby decreasing the organism's ability to adhere to or invade mammalian cells (Ochoa & Cleary, 2009). Wakabayashi *et al*. (2006) reported a protective effect of lactoferrin against infection caused by meticillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans*.

There are several advantageous physiological functions that are attributed to GMP, including (i) binding of cholera and *Escherichia coli* enterotoxins; (ii) inhibition of bacterial and viral adhesion and colonization at intestinal mucosal cells; (iii) suppression of gastric secretions; (iv) support of bifidobacterial growth; and (v) regulation and modulation of immune system responses (Brody, 2000; Choi *et al*., 2012). GMP also has the ability to bind to *Salmonella enteritidis* and enterohemorrhagic *E. coli* O157:H7 (EHEC O157), and its effect is dose dependent. Carbohydrate moieties like sialic acid in GMP are involved in binding to *S. enteritidis* and EHEC O157 (Nakajima *et al*., 2005; Hilde *et al*., 2011).

22.5.1.5 Therapeutic values of whey proteins

GMP is the only naturally occurring protein that does not contain phenylalanine (LaClair *et al*., 2009). The unique amino acid composition, with absence of aromatic residues, such as phenylalanine, histidine, and tryptophan, and the presence of a high proportion of branched-chain amino acids, makes GMP useful for diets aimed at controlling several liver diseases, particularly where branched-chain amino acids serve as a carbon source (Tidona *et al*., 2009; Sharma & Shah, 2010). Lactoferrin can be used as treatment aid for gastrointestinal infections. Di Mario *et al*. (2006) conducted trials with 402 male patients infected with *Helicobacter pylori* and concluded that bovine lactoferrin can be used as an effective adjuvant to 7-day triple therapy for eradication of *H. pylori* infection. Lonnerdal *et al*. (2011) compared the commercially available bovine lactoferrin with human lactoferrin and concluded that bovine lactoferrin is biologically active, and is likely to exert several of the bioactivities of human lactoferrin if added to infant formula. Bovine lactoferrin mediates its anticarcinogenic effects in several ways, including downregulation of cell proliferation, suppression of carcinogen-activating enzymes, inhibition of metastasis, and augmentation of immune system activities (Tsuda *et al*., 2010).

Whey proteins have beneficial roles in cardiovascular diseases. Whey has the possibility to be used as a healthful dietary supplement to reduce blood pressure. Antihypertensive peptides have been isolated in the primary sequence of bovine BLG. These peptides have the ability to restrict constriction of blood vessels. BLG is also believed to have cholesterol-lowering ability (Marshall, 2004). Daily consumption of whey protein-supplemented beverages lowers blood pressure in pre-hypertensive and stage 1 hypertensive young adults. Reports suggest that whey protein beverages can normalize elevated blood pressure and prevent hypertension, which can be a serious health concern in certain persons, especially young women and elderly people (Fluegel *et al*., 2010). In addition to the various advantages, ALA can chelate heavy metals and iron, thereby reducing oxidative stress (Marshall, 2004).

22.5.1.6 Whey proteins in specialized nutrition

22.5.1.6.1 Glycomacropeptide for managing phenylketonuria

Phenylketonuria (PKU) is one of the most frequent inherited metabolic disorders; it is caused by mutations within the gene for phenylalanine hydroxylase (Walter *et al*., 2002). Individuals with PKU lack the enzyme phenylalanine hydroxylase and this results in failure to convert phenylalanine (Phe) to tyrosine in the liver. If the amount of Phe intake is not restricted to below 500 mg/day, they show an increased level of Phe in blood that is toxic to the central nervous system. Such individuals can avoid cognitive impairment and brain damage by eating a highly restrictive diet that limits Phe intake to the minimum amount needed to support growth and protein anabolism (Ney *et al*., 2009).

All natural proteins contain concentrations of Phe that exceed the minimum allowable level for individuals suffering from PKU. Dietary management practices for PKU are focused on restricting all natural proteins that contain Phe. Dietary products for the treatment of PKU have improved; however, they have limitations of poor compliance, especially in adolescents and young adults. Use of GMP, a Phe-free whey protein fraction, is one of the new dietary approaches that improves the palatability and variety of diet for individuals with PKU (van Calcar *et al*., 2009). GMP has a unique amino acid profile suitable for a low-Phe diet because, in its pure form, it is the only known

Table 22.4. Branched-chain amino acid (BCAA) content of some major proteins.

Protein	BCCA content (% w/w of protein)
Whey protein isolate*	26
Egg albumin powder*	22
Milk protein isolate*	20
Soy protein isolate*	17
Human breast milk†	19
Casein†	22
Wheat (whole grain)†	15.4

*Data courtesy of USDEC. Reproduced from Reference manual for U.S. whey and lactose products (www.usdec.org).

†Based on data from Hambraeus (1992).

dietary protein that is naturally free of Phe. Thus, GMP can provide an alternative to amino acid formulae that currently provide the majority of protein in the PKU diet and pose the biggest obstacle to dietary compliance (Ney *et al*., 2009; van Calcar *et al*., 2009). Lim *et al*. (2007) have reported that products made with GMP were acceptable, and Phe-free GMP provides an alternative protein source for individuals with PKU. van Calcar *et al*. (2009) reported that Phe-free GMP is a safe and highly acceptable alternative to synthetic amino acids as the primary protein source in the nutritional management of PKU. Compared with synthetic amino acids, GMP improves protein stability and Phe utilization.

22.5.1.6.2 Whey protein in sports nutrition

For several reasons, athletes need additional protein in their diet. Some of these requirements are to fulfill the increased loss of amino acids oxidized during exercise; to increase the mitochondrial protein content; to provide additional raw materials to replace exercise-induced muscle damage; and to provide supplemental raw materials to enhance muscle protein synthesis (USDEC, 2012). Whey proteins have several characteristics that favor them as an important protein choice for sports nutrition. Whey proteins have high biological values and are easily digested with excellent metabolic efficiency (Hoffman & Falvo, 2004). Concentrated or isolated whey products have a high concentration of protein, a lower concentration of carbohydrates, and a high concentration of essential amino acids and branched-chain amino acids. Whey protein fractions such as ALA and GMP are the richest natural protein sources in terms of the branched-chain amino acids (Table 22.4). Good balance of essential and non-essential amino acids, high concentration of branched-chain amino acids, and low levels of fat and cholesterol make whey proteins superior protein sources for sports nutrition (Etzel, 2004; USDEC, 2012).

During physical exercise, protein synthesis and protein breakdown are stimulated. The balance of protein synthesis and loss determines the anabolic response of muscle exercise. Protein feeding is a simple and effective method to manipulate rates of protein synthesis. The amino acid composition is an important factor that affects protein metabolism during exercise. Essential amino acids are the primary regulators of muscle protein synthesis. However, branched-chain amino acids, especially leucine, are the most important stimulators of skeletal muscle protein synthesis (Volek, 2004; Tunick, 2009). During intense physical exercise, whole body protein synthesis is decreased and proteins are converted into free amino acids. Skeletal muscles have the ability to take branched-chain amino acids from the blood and break them down into glucose for energy. In this way branched-chain amino acids have the ability to provide an energy source during prolonged exercise (Blomstrand *et al*., 2006; USDEC, 2012). Whey proteins are a good source of sulfur-containing amino acids, such as cysteine and methionine (52 mg/g), that maintain antioxidant levels in the body via glutathione synthesis, and are also thought to stabilize DNA during cell division (Marella, 2009). According to the reference manual published by USDEC (2012), there are whey protein bars and whey protein drinks that have been commercialized as sports nutrition products by different companies.

22.5.1.6.3 Whey protein hydrolysates in sports and specialized nutrition

Protein hydrolysates are produced by heat-aided acid or enzymatic hydrolysis of purified protein sources followed by purification processes. Hydrolysis by proteolytic enzymes is greatly preferred because acid hydrolysis oxidizes sulfur-containing amino acids, destroys serine and threonine, and converts glutamine and asparagine to glutamate and aspartate, respectively, thereby lowering protein quality and biological value (Manninen, 2004). A protein hydrolysate is a complex mixture of peptides with varying amino acid chain length together with free amino acids. The availability and size of the peptides depends on the degree of hydrolysis, which indicates the proportions of the peptide bonds that are dissociated from the starting protein molecule (Manninen, 2009). Hydrolysis of major whey protein fractions such as BLG, ALA, and BSA is performed to obtain hydrolysates with high nutritional value and low allergenicity. WPHs also contain the native bioactive peptides, which impart greater advantages to the products (Lourenço da Costa *et al*., 2007; Kurchenko *et al*., 2011). Owing to the breakdown of protein into smaller

peptides, protein hydrolysates are bitter in taste, and the severity of bitterness may depend on the enzymes used, degree of hydrolysis, and specific processing conditions (Leksrisompong *et al*., 2010). However, WPHs are considered to have low bitterness (Guadix *et al*., 2006). Ingredia (France), Peptogen (Denmark) and Hilmar (USA) are some of the companies presently involved in commercial production of milk and WPHs (Kurchenko *et al*., 2011).

Short-chain peptides resulting from protein hydrolysis have antioxidant activity and may have potential as natural antioxidants in food products. Certain amino acid residues in the peptides, such as tyrosine, methionine, histidine, lysine, and tryptophan, have the ability to chelate metal ions, thereby protecting lipid oxidation (Peña-Ramos & Xiong, 2001). WPHs have been used in sports and specialized nutrition (Kurchenko *et al*., 2011). It has been reported that protein hydrolysates providing mainly dipeptides and tripeptides are superior to intact proteins and free amino acids in terms of skeletal muscle protein synthesis. Ingestion of protein hydrolysate enhances blood amino acid levels, muscle protein anabolism, exercise performance, and muscle glycogen resynthesis (Manninen, 2004, 2009; Kurchenko *et al*., 2011). WPHs are also considered to be ideal ingredients in the formulation of human milk substitutes due to their high nutritional value, low bitterness, and low antigenicity (Guadix *et al*., 2006).

22.5.1.6.4 Whey proteins in diets for obesity

Obesity remains a serious issue in the USA and thus diets low in carbohydrate and high in protein are favored in obese individuals. For such diets, whey is an attractive source of dietary protein (Pordesimo & Onwulata, 2009). WPIs contain as much as 95% protein, after the removal of fat and lactose, and valuable minerals and vitamins. There is a significant commercial impact of whey in the weight-loss diet for its protein content alone. On consumption of whey-rich diets, the essential and non-essential amino acids in whey act as substrates for protein synthesis and improve body mass index (Marshall, 2004).

Whey is an inexpensive source of high nutritional quality protein. Utilization of whey as a physiologically functional food ingredient for weight management is of increasing interest for nutritionists. The role of individual whey proteins and peptides in regulating body weight has not been fully defined (Pordesimo & Onwulata, 2009). However, it has been reported that whey protein reduces short-term food intake. The satiety effect of whey protein is probably due to the actions of individual whey protein fractions, bioactive peptides, amino acids released after digestion, or the combined action of whey proteins, peptides, amino acids, and other milk constituents (Luhovyy *et al*., 2007).

The functionality of whey proteins in the diet for obese individuals is related to stimulation of cholecystokinin (CCK) by oral intake of GMP. CCK is known to be an important satiety hormone, which may make whey protein a useful component of a weight-loss diet. It decelerates gastric emptying, which may in turn support satiety (Keogh *et al*., 2010). Research has shown that WPC is significantly more effective than red meat in reducing body-weight gain and body fat content of laboratory rats while net energy intake is maintained comparably. It has also been reported that GMP is associated with reduced fat mass in Wistar rats fed diets of different protein types and amounts (Royle *et al*., 2008). On an energy basis, protein appears more satiating than the other macronutrients. Studies have shown that protein leads to long-term weight loss. A higher protein intake has also been shown to limit weight regain after weight loss. A high-protein weight-loss diet causes a greater reduction in total and abdominal fat in women (Keogh & Clifton, 2008). Luhovyy *et al*. (2007) have described insulin, glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide, peptide YY, and ghrelin to be the different satiety hormones associated with whey protein products.

22.5.2 Lactose

Dried whey contains an abundant quantity of lactose. Lactose undergoes slow hydrolysis during digestion and generates a prolonged energy supply (Vesa *et al*., 2000). Owing to its slow absorption, lactose has a slight laxative effect mainly due to the lowering of pH, which causes increase in intestinal peristalsis (Renner, 1983). Glucose and galactose, the monomers of lactose hydrolysis, are actively absorbed from the intestine (Renner, 1983). In general, carbohydrates increase intestinal calcium absorption, and lactose contained mainly in dairy products seems to be the most effective carbohydrate supporting calcium absorption. Lactic acid, the metabolic product of lactose, lowers the pH in the intestine, which increases the solubility of calcium making it more available for absorption (Pérez *et al*., 2008). Lactose also possesses prebiotic characteristics and stimulates the growth of lactic acid bacteria in the intestinal tract, thereby favoring their probiotic activity. The low pH due to lactose metabolism in the intestine inhibits proteolytic organisms and helps establish the probiotic organisms (Szilagyi, 2002).

22.5.2.1 Whey products for lactose intolerance

Deficiency of the enzyme lactase in the gastrointestinal tract is why many people of ethnic minority populations around the world are unable to digest lactose, and this phenomenon is described as lactose intolerance or lactose

Table 22.5. Vitamin content in sweet whey powder.

Vitamin	Concentration (per gram)
Thiamin B_1	0.5 mg
Riboflavin	2.2 mg
Niacin	1.3 mg
Pantothenic acid	5.6 mg
Vitamin B_6	2.4 µg
Folic acid	12.0 µg
Vitamin B_{12}	2.4 µg

Source: data courtesy of USDEC. Reproduced from Reference manual for U.S. whey and lactose products (www.usdec.org).

maldigestion (Yang & Silva, 1995). Whey powder contains substantial amounts of lactose and thus official recommendations (Department of Health and Human Services, 2011) advise that it should be avoided by lactose maldigesters (Lomer *et al*., 2008). Reports suggest that many lactose maldigesters tolerate small to moderate amounts of lactose without significant digestive issues. Consumption of liquid dairy products with reduced lactose content will benefit some individuals (Vesa *et al*., 2000). Reports suggest that milk intolerance and lactose intolerance are different phenomena. In one study, only 33% of African Americans with lactose maldigestion claimed symptoms after ingestion of a moderate amount of milk (Johnson *et al*., 1993). From this result it can be inferred that WPCs with high concentrations of protein and lower concentrations of lactose may not be a problem for lactose maldigesters.

22.5.3 Vitamins and minerals in whey

All the water-soluble vitamins in milk remain in the serum portion during coagulation and are collected with whey. During processing of whey, the concentration of vitamin C is reduced or lost, and whey is not considered a source of vitamin C. Whey powders contain higher concentrations of other water-soluble vitamins (Table 22.5), and when incorporated into food products, whey products will naturally fortify the thiamin, riboflavin, pantothenic acid, vitamin B_6, and vitamin B_{12} contents (USDEC, 2012).

Calcium, zinc, magnesium, and phosphorus are the major minerals available in whey powders. Mineral content is higher in acid whey than in sweet whey. A sample of 100 g of acid whey contains 2054 mg of calcium, 6.31 mg of zinc, 199 mg of magnesium, and 1348 mg of phosphorus, while in sweet whey powder the concentration of these minerals is 796, 1.97, 176, and 932 mg, respectively (USDEC, 2012).

22.6 FUTURE PROSPECTS FOR DIETARY APPLICATIONS OF WHEY

Whey products of different functional and nutritional significance are available on the market. Technologies are available for commercial production of individual constituents present in whey. Increased production of whey ingredients has resulted in an increase in the application of whey products in nutritional, therapeutic, and functional usages. Whey proteins easily fit into several food formulations such as confectionary, beverages, baked foods, desserts, convenience foods, foods for athletes, foods for elderly people, foods for infants, and many other specialized nutrition formulations (Onwulata, 2009). Whey products also have increased prospects as ingredients in clinical and pharmaceutical applications. High concentrations of both essential and non-essential amino acids, minerals, and biologically active proteins in whey should encourage extensive application in clinical nutrition, particularly for post-surgical wound healing (Marshall, 2004). High concentrations of sulfur-containing amino acids, particularly cysteine and its role in the synthesis of glutathione, are believed to be beneficial to individuals infected with HIV. Whey provides a higher concentration of intact native proteins such as lactoferrin and immunoglobulins, which are being used for immune modulation studies.

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23
Goat Milk

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23.1 INTRODUCTION

Goats were the first species to be domesticated as livestock about 8000 BC in Mesopotamia, part of today's Middle East. For centuries, humans have used goats for many purposes in all continents. However, the goat sector has not been well supported worldwide compared with other animal production sectors, especially the bovine milk sector, despite the fact that in the last few decades the goat has emerged as one of the major livestock species, rising in numbers compared with others (Zervas & Tsiplakou, 2011).

More than any other mammalian farm animal, the goat is a main supplier of dairy, meat, and fiber products for rural people, and is regarded as an important economic part of utilizing arid and semi-arid lands through farming of less favored areas worldwide, adapting well to many different climates, geological and management conditions, and in many cases being irreplaceable by any other livestock. Goats can utilize pasture and forage that cattle find difficult to consume. For these reasons, the old saying that "the goat is the cow of the poor people" is true. In developing countries, the production of goat milk is a useful strategy to tackle the problem of undernutrition, especially among the infant population (Haenlein, 2004). The use of dairy goats in livestock interventions in sub-Saharan Africa and Malawi, where few or no infant formulae exist for HIV-positive mothers in poor rural households, is an alternative option. Mothers with HIV/AIDS are advised to exclusively breastfeed their babies in the first 6 months because this period has a lower risk for transmission of the virus to the child (UNICEF, UNAIDS, WHO & UNFRA, 2003). After 6 months, mothers are advised to stop breastfeeding and use affordable, acceptable, available, safe, and sustainable milk replacements: goat milk is a valuable option.

Goats constitute natural renewable resources, very diverse in terms of genetic potential, distribution, function, and productivity, and are the most efficient transformers of low-quality forage into high-quality animal products with distinct chemical composition and organoleptic characteristics.

The present total world population of 921 million goats is found mainly in areas with temperate pasture-growing conditions (Devendra, 2012). It is estimated that Asia and Africa together account for as much as 91.5% of the world's total goat population, while the corresponding figure for Oceania and Europe together is 2.4%. Goat milk production represents about 2.2% of total world milk production (Zervas & Tsiplakou, 2011), while in 2009 sheep milk comprised 1.3% and camel milk 1.3% (Seifert, 2012). However, on a world basis, more people drink goat milk than milk from any other single species, despite the fact that dairy cows produce the greatest amount of the world's milk, mostly in developed countries (Park & Haenlein, 2007). According to Food and Agriculture Organization data for 2009, 59% of world goat milk production was produced in Asia, 21% in Africa, and 16% in Europe (Seifert, 2012). Goat milk production has been increasing during recent years: in 2010 globally by 0.2%, with the greatest increases in France (6.4%) and Turkey (3.5%), but decreases in The Netherlands (–8.6%), Spain (–2.9%), and Mexico (–1.0%). According to current US

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Department of Agriculture data, the USA has 2.86 million head of goats with 360 000 milk goats (Harman, 2012).

Dairy goat farming is a vital sector of agricultural business in developed countries of the Mediterranean region, where 16% of the world's goat milk is produced (Pandya & Ghodke, 2007). In countries like France, Italy, Spain, and Greece, almost all goats belong to dairy breeds; milk is the main product and kid's meat considered a by-product. This fact may prove that goat dairying is not necessarily synonymous with underdevelopment and poverty (Haenlein, 2004). On the contrary, cheese production from goat milk is a significant industry in France and Italy and regarded as gourmet food commanding high prices.

23.2 COMPOSITION OF GOAT MILK

Information on composition and physicochemical characteristics of goat milk is essential for successful development of the dairy goat industry and for the marketing of the products. There are unique differences in several important constituents and physical parameters of milk, such as proteins, lipids, minerals, vitamins, carnitine, glycerol ethers, enzymes, fat globule size, and casein polymorphisms, between goats, cows, and sheep; the composition of milk macronutrients and micronutrients produced by a given species can be influenced by several factors. Table 23.1 shows the average composition of milk from goats, sheep, cows and humans. These compositional figures are only average values and do not represent individual animals, since considerable differences exist between breeds and among individual animals. Milk composition varies according to genotype, individuality, stage of lactation, parity, season, feeding, management, reproduction, and sanitary characteristics of animals, locality, and socioeconomic environment (Park *et al*., 2007). These are the reasons for deviations in compositional data for goat milk presented by different authors. In addition, Morgan *et al*. (2003) determined a high level of variability in biochemical composition, bacteriological quality, and technological properties of goat milk collected during 1 year from different European countries (Greece, Portugal and France).

Table 23.1. Average composition (g/kg) of goat, sheep, cow and human milk, showing main constituents.

Composition (g/kg)	Goat	Sheep	Cow	Human
Total solids	119.4	190.0	128.9	127.4
Fat	38.0	79.0	36.0	40.0
Lactose	41.0	49.0	47.0	69.0
Protein	34.0	62.0	33.0	12.0
Casein	25.0	42.0	26.0	4.0
Albumin, globulin	7.0	10.0	6.0	7.0
Non-protein nitrogen	4.0	8.0	2.0	5.0
Ash	8.0	9.0	7.0	3.0
kcal/dL	70.0	105.0	69.0	68.0
Cholesterol	0.10	0.11	0.13	0.16

Sources: based on data from Park *et al*. (2007) and Slačanac *et al*. (2010).

In local breeds, not bred for high milk production, milk composition is often similar to that of sheep, having a very high dry matter (DM) content (135–175 g/kg), fat (45–65 g/kg), and crude protein (40–55 g/kg). Dairy breeds like Saanen, Alpine, and Toggenburg with high milk yields give milk that is low in DM (110–135 g/kg), often due to low levels of fat (30–40 g/kg) and crude protein (27–35 g/kg) (Morand-Fehr *et al*., 1991). Thus, goat milk from such dairy breeds is apparently less suitable for cheesemaking due to low levels of useful constituents, caseins in particular. Morand-Fehr *et al*. (1982) have shown that milk from dairy breeds of cows and goats is very similar in terms of composition of its DM, which is less true for the milk from local breeds. Goat, sheep, and cow milk contain substantially higher levels of protein, casein, and ash than human milk (Table 23.1). The fat in goat milk, percentage wise, is similar to cow milk, but the physical and chemical structures are significantly different between the two species.

The quality of milk can be evaluated by various criteria such as dietetic, nutritional, sanitary, and technological. These parameters are mainly linked to its main components (fat, protein, lactose) and to their physicochemical characteristics as well as to micro-components: minerals, vitamins, minor fatty acids, conjugated linoleic acid (CLA), cholesterol, terpenes.

23.2.1 Fat

Fat content is the most variable component of milk, quantitatively and qualitatively, in terms of cost, nutrition, physical and sensory characteristics; it impacts dairy products depending on lactation stage, season, breed, genotype and feeding. The characteristics of goat milk fat that have important consequences for manufacturing are:

1. the size of fat globules, which is smaller in goat milk compared with cow milk (Jenness, 1980); and
2. the fatty acid profile of goat milk, which contains a higher proportion of short-chain fatty acids (SCFAs) (Haenlein, 1992).

In both species the fat globules range from 1 to 10 μm, but the number of fat globules smaller than 5 μm is 60% in cow milk, whereas it is about 80% in goat milk. This difference results in softer texture of goat milk products, though it

makes manufacture of butter from goat milk more difficult (Silanikove *et al*., 2010). Apart from their smaller diameter, the fat globules in goat milk are better distributed in the milk–lipid emulsion than the fat globules in bovine milk. The smaller fat globules of goat milk are better dispersed, being naturally homogenized, and a provide a greater surface area of fat for better human digestion by lipases.

23.2.2 Fatty acids

The three main SCFAs, caproic (C6:0), caprylic (C8:0), and capric (C10:0), which are positively related to the "goaty" flavor intensity in goat milk, form up to 15–18% of goat milk fat fatty acids, but only 5–9% in cow milk (Chilliard *et al*., 2006). Comparative fatty acid profiles for goat versus sheep (Table 23.2) and goat versus cow milk have been reported (Park *et al*., 2007; Sanz Ceballos *et al*., 2009; Slačanac *et al*., 2010; Zervas & Tsiplakou, 2011). Sanz Ceballos *et al*. (2009) found higher proportions of SCFAs (C6–C14) in goat milk fat than in cow milk, but also higher proportions of *n*-3 and *n*-6 polyunsaturated fatty acids (PUFAs), with a markedly lower ratio of *n*-6 to *n*-3 in goat milk. Haenlein (2004) and Park *et al*. (2007) reported that goat milk fat has a higher content of monounsaturated fatty acids (MUFAs). Similar to SCFAs, Haenlein (1992) emphasized the beneficial proportions of MUFAs and PUFAs for human health, especially for cardiovascular diseases.

CLA has gained great attention in recent years because of its beneficial effect on health. The content of CLA in goat milk is usually higher than in cow milk, which may be due to the semi-intensive nature of the system under which goats are normally farmed. Comparing sheep with goats, when sheep and goats were fed indoors with the same diet, sheep milk had higher CLA and vaccenic acid contents than goats (Tsiplakou & Zervas, 2008a). Also, when olive tree leaves with high C18:3 content, or grape marc with high C18:2 content were included in sheep and goat diets, their response, as far as CLA milk content is concerned, was different (Tsiplakou & Zervas, 2008b), favoring sheep. These different responses of sheep and goats in milk CLA and fatty acid profile, under the same dietary treatments, show possible species differences that could be explained by the differences found in the mRNA of stearoyl-coenzyme A desaturase of their mammary adipocytes (Tsiplakou *et al*., 2009). Furthermore, it has been found that the *trans*-10, *cis*-12 CLA isomer did not increase above trace levels in goat milk, even when *trans*-10 C18:1 increased and did not inhibit milk fat secretion after rumination when infused in goats (Andrade & Schmidely, 2006), in contrast to cows (Griinari & Bauman, 2003).

Branched-chain fatty acids (BCFAs) have also been found in goat milk by Alonso *et al*. (1999), which lend characteristic flavors to dairy products. Most cholesterol in goat milk, as in cow milk, is in a free state (52 mg/100 g fat), with a small portion in ester forms, which constitutes less than 4% of the total cholesterol (Jenness, 1980; Chandan *et al*., 1992).

Table 23.2. Average (and minimum/maximum) fatty acid profile (% of total fatty acid methyl esters) of sheep and goat milk fat.

	Sheep milk fat		Goat milk fat	
Fatty acid	**Mean**	**Min./max.**	**Mean**	**Min./max.**
C4:0	3.51	3.07–3.93	2.18	1.97–2.44
C6:0	2.90	2.68–3.44	2.39	2.03–2.70
C8:0	2.64	2.10–3.27	2.73	2.28–3.04
C10:0	7.82	5.54–9.73	9.97	8.85–11.0
C10:1	0.26	0.23–.31	0.24	0.19–0.38
C12:0	4.38	3.48–4.92	4.99	3.87–6.18
C12:1	0.04	0.03–0.05	0.19	0.10–0.40
C13:0	0.17	0.13–0.22	0.15	0.06–0.28
C14:0	10.4	9.58–10.7	9.81	7.71–11.2
iso-C15:0	0.34	0.26–0.43	0.13	0.12–0.15
anteiso-C15:0	0.47	0.33–0.60	0.21	0.17–0.24
C14:1	0.28	0.19–0.50	0.18	0.17–0.20
C15:0	0.99	0.89–1.11	0.71	0.46–0.85
iso-C16:0	0.21	0.17–0.26	0.24	0.17–0.40
C16:0	25.9	22.5–28.2	28.2	23.2–34.8
iso-C17:0	0.53	0.44–0.59	0.35	0.24–0.52
anteiso-C17:0	0.30	0.26–0.36	0.42	0.30–0.50
C16:1	1.03	0.74–1.27	1.59	1.00–2.70
C17:0	0.63	0.58–0.70	0.72	0.52–0.90
C17:1	0.20	0.17–0.22	0.39	0.24–0.48
C18:0	9.57	8.51–11.0	8.88	5.77–13.2
C18:1 total	21.1	17.8–23.0	19.3	15.4–27.7
C18:2 total	3.21	2.89–3.57	3.19	2.49–4.34
C20:0	0.45	0.36–0.52	0.15	0.08–0.35
C18:3	0.80	0.52–1.04	0.42	0.19–0.87
C18:2 conjugate total	0.74	0.56–0.97	0.70	0.32–1.17

Source: reproduced from Park *et al*. (2007), with permission of Elsevier.

23.2.3 Proteins

Goat milk has six principal proteins, β-lactoglobulin (BLG), α-lactalbumin (ALA), κ-casein, β-casein, α_{s1}-casein, and α_{s2}-casein, which are about the same as in cow milk (Park *et al*., 2007), but they differ in genetic polymorphisms and their frequencies in goat populations (Martin, 1993). The quantitative characteristics of the

Table 23.3. Quantitative characteristics of the protein fraction of milk (in g/L) from different dairy goat breeds.

	Total protein	κ-Casein*	α_{s2}-Casein*	α_{s1}-Casein*	γ-Casein*	β-Casein*	Total casein*	ALA*	BLG*
Indigenous Greek breed									
N	56	56	56	56	56	56	56	56	56
Mean	38.8*	3.93	3.93	6.90*	1.51	13.2*	29.5*	2.49*	3.35*
SD	2.93	0.72	0.86	1.57	0.31	1.18	2.33	0.61	0.65
Min.	33.1	2.50	1.57	3.62	0.94	10.9	24.8	1.51	2.22
Max.	46.8	5.56	6.14	9.58	2.12	16.2	34.5	4.38	5.63
International breeds									
N	60	60	60	60	60	60	60	60	60
Mean	31.9†	3.77	3.84	3.02†	1.37	11.5†	23.5†	2.25†	2.94†
SD	3.5	0.59	0.68	2.35	0.30	1.42	3.17	0.44	0.64
Min.	24.6	2.65	1.67	0	0.74	8.42	15.8	1.40	1.59
Max.	43.5	5.29	5.47	7.69	2.2	15.0	31.4	3.26	4.83

The corresponding means with different superscripts were significantly different (*t*-test at the 95% confidence level).
*Estimated by the area of the chromatographic peaks and by the compositional data.
† Estimated by Milkoscan.
Source: reproduced from Moatsou *et al*. (2008), with permission of Elsevier.

Table 23.4. The main casein fractions (%) in goat and cow milk.

Casein fraction	Goat milk	Cow milk
α_s-Casein	26	56
β-Casein	64	33
κ-Casein	10	11
α_s-Casein/β-casein	0.41	1.70

Source: reproduced from Slačanac *et al*. (2010), with permission of John Wiley & Sons.

protein fraction of milk from different goat breeds are presented in Table 23.3. Casein is the basic protein in milk, constituting about 80% of total milk proteins. Casein micelles in cow milk are small (60–80 nm) compared with those in goat milk, which range between 100 and 200 nm. Another significant difference between the two species is the level of α_{s1}-casein, which in goat milk ranges from 0 to 7 g/L. The variability is associated with polymorphisms within the α_{s1}-casein gene, which is common in goats (Clark & Sherbon, 2000). The percentages of the main casein fractions in goat and cow milk are presented in Table 23.4. Goat milk has markedly different levels of α_{s1}-casein and α_{s2}-casein from those found in cow milk. Goat milk has much lower (or no) α_{s1}-casein and higher α_{s2}-casein contents than cow milk, which may have none. The variability of the casein ratios in caprine milks, in particular the ratio of α_{s1}-casein to α_{s2}-casein, can affect micelle structure and possibly calcium availability. Cheeses from normal cow milk have a ratio of α_{s1}-casein to α_{s2}-casein of 5 : 1, whereas that of goat milk is 3 : 1. β-Casein is the major component of the casein fraction in goat milk, whereas α_{s1}-casein is the major casein in cow milk. Lamothe *et al*. (2007) found that the extraction yield of β-casein was 53% higher in goat than in cow milk. The relative absence of α_{s1}-casein and the greater proportion of β-casein make goat milk closer to human milk. β-Casein has a very important impact on the structural and nutritive differences between goat and cow milk (Haenlein, 2004). Goat milk proteins are more digestible than those of cow milk, which may be due to the high levels of α_{s2}-casein in goat milk (Haenlein, 2004; Park *et al*., 2007).

23.2.4 Whey proteins

Goat milk serum contains mainly albumin, BLG and ALA. ALA levels in goat milk serum, according to Jenness (1980), are nearly twice as much as those in cow milk, while according to Storry *et al*. (1983) its content is approximately the same in the two dairy animal species. BLG content in goat milk is practically double that of ALA (Park & Haenlein, 2007).

23.2.5 Amino acids

The total amino acid content in goat and other non-primate milks is substantially greater than that in human and primate milk. The essential amino acid content of goat and cow milk is greater than that of human milk, whereas the

opposite trend is observed for the branched-chain amino acid content (Davis *et al*., 1994). A comparative average amino acid profile of goat and cow milk proteins is presented by Haenlein (2004). The α_s-casein contains more aspartate, lysine, and tyrosine than β-casein, whereas β-casein has more leucine, proline, and valine content than α_s-casein. BLG contains significantly less aspartate than ALA, whereas the opposite trend is noticed for alanine and glutamate concentrations (Davis *et al*., 1994).

In goat milk, the free amino acid taurine, derived from sulfur-containing amino acids, has important metabolic functions as does carnitine, which is a valuable nutrient for the human neonate. It is involved in the formation of the infant brain and of bile salts, calcium flux, antioxidant activity, neuron excitability, and stabilization of membranes (Redmond *et al*., 1998).

23.2.6 Non-protein nitrogen

Non-protein nitrogen (NPN) content in goat and human milk is much higher than in cow milk, but in goat and cow milk it differs between breeds (Park, 1991). The NPN content is one of the reasons why goat milk has been identified as a "healthy" milk (Slačanac *et al*., 2010).

23.2.7 Minor proteins

Goat milk has similar levels of immunoglobulins, ferritin, lactoferrin, transferrin proteose peptone, calmodulin, folate-binding protein, and prolactin to those of cow milk. Human milk contains more than 2–50 mg/mL of lactoferrin, levels which are 10- to 100-fold higher than in goat milk and cow milk, which contain transferrin levels of 20–200 mg/mL (Park, 2009). Table 23.5 presents the goat milk content of minor proteins, enzymes, and other constituents (Park & Haenlein, 2007).

Table 23.5. Concentrations of minor proteins, enzymes and other constituents in goat milk.

Constituent	Concentration range
Lactoferrin	20–200 μg/mL
Transferrin	20–200 μg/mL
Prolactin	44 ng/mL
Folate-binding protein	12 μg/mL
Lysozyme	25 μg/dL
Ribonuclease	425 μg/dL
Lipase	36 μmol/dL
Lactate dehydrogenase	47 μmol/s per mL
Malic dehydrogenase	50 μmol/s per mL
Xanthine oxidase	19–113 μL O_2/h per mL
Alkaline phosphatase	11–13 mg/L
Orotic acid	13 mg/L
Carnitine	16.4 mg/L
ATP	19 mg/L
Free amino acid	48 mg/L
Sialic acid protease	13.89 mg/L

Source: reproduced from Park & Haenlein (2007), with permission of John Wiley & Sons.

23.2.8 Carbohydrates

Lactose is the major carbohydrate in goat, as in cow, milk, but at a lower level (Table 23.1). Lactose concentration does not vary excessively. However, goat milk lactose content is increased by dietary plant oil supplementation in contrast to cow milk (Chilliard *et al*., 2005). Lactose favors the absorption of calcium, phosphorus, and magnesium and the utilization of vitamin D in human digestion. Goat milk is significantly richer in lactose-derived oligosaccharides than cow milk. Milk oligosaccharides are thought to be beneficial for human nutrition due to their probiotic and anti-infective properties (Martinez-Férez *et al*., 2006).

23.2.9 Minerals and vitamins

The mineral content of goat milk varies from 0.7 to 0.85%. Compared with human and cow milk, goat milk contains more Ca, P, Mg, K, and Cl, and less Na and S. Their levels, as those of trace elements, can vary depending on the breed, diet, animal, and stage of lactation (Park, 2006). However, goat milk has lower Fe, and higher Zn and I contents than human milk (Park, 2009).

The vitamin content of goat milk is similar to that of cow and human milk. Goat milk supplies adequate amounts of vitamin A, niacin, thiamin, riboflavin, and pantothenate for human infants (Park, 2009), whereas it is poor in folic acid and vitamin B_{12} (Park & Haenlein, 2006). Goat milk lacks β-carotene, which is entirely converted into retinol and is the reason why goat milk is always white in contrast to cow milk.

23.3 EFFECTS OF FEEDING AND MANAGEMENT ON GOAT MILK COMPOSITION

Goat milk is much used for cheesemaking and while goat farmers generally aim to increase milk output in quantitative terms, they are equally concerned in maintaining or improving its composition. If they try to make use of genetic factors by breeding, it will take several years' work until they achieve visible results. Thus, the alternative is to use dietary factors and herd management methods, over which farmers have at least better and more direct control and which can often have an effect on milk composition within 1 or 2 days. Furthermore, the higher the milk output of the goat, the greater the impact of diet on milk composition (Morand-Fehr *et al*., 1991).

The potential to alter milk composition by feeding has been investigated, but the results show high coefficients of variation (CVs) for fat content but low CVs for protein and casein content. The variation in milk fat composition is very large, with important interactions between forage, concentrates, and oils in almost all major and minor fatty acids. The aim is to improve the effects of different feeding strategies to selectively increase those fatty acid that are of interest for human nutrition and health without increasing the less desirable fatty acids and without decreasing the sensory quality of dairy products.

Lipid supplementation of goat diets increases the milk fat content in most cases and allows much less saturated fatty acid, much more oleic and/or vaccenic and CLA, and more C18:3 and other *trans* fatty acids (Sanz Sampelayo *et al*., 2007). There is also an effect of stage of lactation, since an increasing response in milk fat content and C18:0 fatty acid secretion in late lactation of goats has been observed (Bernard *et al*., 2005). The productive capability and stage of lactation of the animal should also be taken into account (Fig. 23.1, see also Plate 23.1).

The physical form in which the forage proportion of the diet is present and/or the forage–concentrate ratio in the diet are aspects for special consideration in determining the fat content of milk. The scope of the effect depends on the nature and amount of the dietary fat and on the interactions between the basal diet (e.g. forage type, fiber amount and length, starchy concentrate).

Milk from goats grazing pasture is naturally enriched in fat-soluble vitamins, terpenes, unsaturated fatty acids, and CLA compared with those fed conventional forage plus concentrate diets (Galina & Haenlein, 2004; Chilliard *et al*., 2005; Tsiplakou *et al*., 2006). In recent years, consumers have demanded healthier food and have paid increasing attention to milk fatty acid profiles and cholesterol content, which have important repercussions on human health. The grazed forage contains many naturally occurring bioactive molecules with antioxidant and anti-inflammatory properties, such as bioflavonoids and phytosterols, as a result of multi-plant species composition and xerothermic climatic conditions, and these compounds are transferred to milk and dairy products. Most browsing animal species such as goats like fodder trees, fodder shrubs and herbaceous species, which contain large amounts of polyphenols. Many of them have tanniferous components that constitute 50–80% of the forage selected by goats all year round. This milk is rich in microcomponents (fatty acids, vitamins), volatile compounds (flavonoids, terpenes), and phenolic compounds favorable to human nutrition and health, with higher oxidative stability, processing efficiency, and quality (Silanikove *et al*., 2010). Cuchillo-Hilario *et al*. (2010) showed that soft goat cheese antioxidant activity can be modified by the feeding system. Grazing management represents a better option than indoor feeding, producing a healthy profile of bioactive compounds that provide increased concentrations

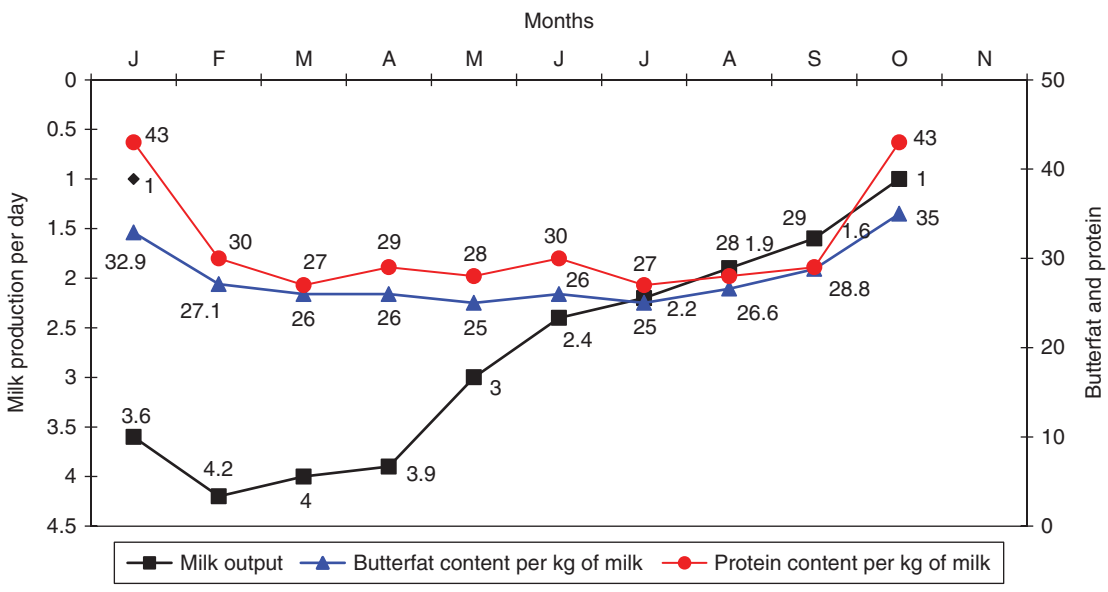


Figure 23.1. Changes in milk yield (kg) and butterfat and protein content (g/kg) during different stages of lactation in goats. Modified from Le Jaouen (1987), with permission of New England Cheesemaking Supply Company (www.cheesemaking.com). For a color version of this figure, see Plate 23.1.

of total polyphenols, hydroxycinnamic acids, and flavonoids. In addition, the majority of goat farms are extensively managed, making use of feed resources locally available without buying feed additives, antibiotics or other pharmaceuticals for disease prevention; also, the rangelands used have not been treated with artificial fertilizers or agrochemicals (Zervas, 1998). The effect of feeding on goat milk composition has been extensively reported by Zervas and Tsiplakou (2011). Indoor intensive feeding management with zero grazing involves elevated housing with slatted wooden or expanded corrugated floors, especially in tropical countries like Taiwan (100% of herds of 500 000 head of Alpines and Saanens) or the Philippines, and this elevated housing type satisfies ventilation needs, provides effective internal parasite control because of the slatted floors, and enables sales of the manure collected from underneath the elevated housing (Haenlein, 1985; Goddard, 2012). The higher cost of such housing is expected to be recovered quickly from improved milk quality and higher yields (Yogendran, 2012).

23.4 THE CONTRIBUTION OF GOAT MILK TO HUMAN NUTRITION AND HEALTH

Milk is a source of nutrients for neonates of mammalian species, and children and adult humans use it for growth and nourishment. It is often considered as a functional food, since it contains varieties of different bioactive components, such as casein and whey proteins, which have been found to be increasingly important for physiological and biochemical functions that have crucial impacts on human metabolism and health (Gobbetti *et al*., 2007). Today, goat milk is of particular interest because of its specific composition, which has led to it being considered a high-quality raw material for manufacturing food for infants and elderly people, as well as for certain sectors of the population with particular needs (Haenlein, 1992, 2004; Park, 2006). Goat milk is becoming more relevant for the human diet because of its nutritional value, digestibility, and therapeutic and dietary characteristics (Raynal-Ljutovac *et al*., 2008). Thus goat milk can also be essential in infant formulae (Bouckenooghe *et al*., 2006).

Even though nutritional interest in goat milk and its significance in human nutrition have been recognized for years, very few technical studies have been conducted and published in peer-reviewed journals or technical books. However, a number of studies have identified some bioactive compounds in goat milk and dairy products with specific biochemical, physiological, and nutritional functionalities and characteristics that have strong potential for beneficial effects on human health, such as:

- gastrointestinal development, activity and function;
- infant development;
- immunological development and function; and
- microbial activity, including antibiotic and probiotic action (Gobbetti *et al*., 2007).

Because of its unique characteristics, such as high digestibility, distinct alkalinity, greater buffering capacity, higher content of SCFAs, medium-chain fatty acids (MCFAs), Zn, Fe, Mg, and Ca, stronger lactoperoxidase (antimicrobial) activity, better immunological and antibacterial characteristics, and high levels of some amino acids (e.g. valine, glycine and hystidine), goat milk has been recommended as a nutritionally healthy and therapeutically functional food source for patients suffering from various allergies, and for those who need better nutrient absorption, greater nutrient bioavailability, and higher buffering capacity for ulcer treatment (Park, 1994; Slačanac *et al*., 2010).

Human milk is the best milk for infants. When breastfeeding is not possible, the best alternative is an infant formula that has been adjusted to meet the nutritional needs of the infant. Goat milk and cow milk are both sources of high-quality fat, protein, minerals, and some vitamins, but the milk secretion processes in these two mammals vary, giving rise to important compositional differences. The secretory process in the goat is more like the secretory process in humans. Goat milk is consequently more similar to human milk than cow milk in some respects, making it a logical and valuable alternative to cow milk, which is the most commonly used starting material for the production of human milk substitutes.

Mack (1953) reported that children fed goat milk showed significantly greater nutrient bioavailability and growth parameters than those fed cow milk. When Grant *et al*. (2005) studied 72 newborns for 168 days, they observed a non-significant higher weight gain in infants ($N=36$) fed goat milk-based infant formula than in those ($N=36$) fed cow milk-based infant formula, while both formulae studied had similar nutritional composition in terms of energy and composition of macronutrients.

23.4.1 The effects of milk fat

The biomedical superiority of goat milk and its products, as far as fat composition is concerned, has not been much promoted in marketing, but has great potential in justifying the uniqueness of goat milk in human nutrition and medicine for treating various gastrointestinal disorders and diseases besides its value in alleviating cow milk allergies (Haenlein, 1992, 2004). Goat milk fat may be more rapidly

digested than cow milk fat because lipase attacks ester linkages of SCFAs or MCFAs more easily than those of longer chains (Jenness, 1980; Park, 1994). López-Aliaga *et al*. (2010) presented the effects of goat and cow milk fat on digestive utilization of fat and on some of the biochemical parameters related to the metabolism of lipids in malabsorption syndrome. The consumption of a goat milk-based diet for 14 days improved digestive utilization of fat and reduced fecal losses compared with cow milk, and it approached the values obtained using olive oil. Also, in an Algerian study of infants with malabsorption syndrome, the substitution of goat milk for cow milk caused higher rates of intestinal fat absorption (Hachelaf *et al*., 1993). The beneficial effects of dietary inclusion of goat milk on the utilization of protein, fat, and minerals (Ca, P, Mg, Fe, Cu, Zn, and Se), which affect malabsorption syndrome caused by resection of the intestine, have been reviewed by López-Aliaga *et al*. (2010), who concluded that goat milk, as an alternative to cow milk, can be an excellent natural food in cases of malabsorption syndrome.

Goat milk contains a higher proportion of SCFAs and MCFAs than cow milk and these contribute significantly to human nutrition (Wong *et al*., 2003). MCFAs are absorbed intact and do not undergo the degradation and re-esterification process. They are more readily hydrolyzed by pancreatic enzymes than long-chain fatty acids (LCFAs), providing energy in growing children. Thus, the use of MCFAs as an adjunct to weight loss has been a further aspect of interest, because MCFAs constitute a rapid energy supply, especially for subjects suffering from malnutrition or fat malabsorption syndrome. MCFAs have been used since 1960 for preterm newborns in a specific ratio with LCFAs (Telliez *et al*., 2002). Owing to this favored pathway, they may contribute to lower total circulating cholesterol and especially low-density lipoproteins (LDLs) (Kasai *et al*., 2003). According to Haenlein (1996) the medical and pediatric literature has documented the treatment benefits of SCFAs in cases of malabsorption syndrome, premature infant feeding, cholesterolemia, gallstones, and cystic fibrosis. In addition, goat milk fed to infants or children with digestive malnutrition has being found to be an equal or even superior substitute to cow milk (Razafindrakoto *et al*., 1993; Alferez *et al*., 2001). SCFAs have also been used therapeutically for treating patients with malabsorption suffering from steatorrhea, chyluria, hyperlipoproteinemia, intestinal resection, coronary bypass, and childhood epilepsy (Greenberger & Skillman, 1969; Tantibhedhyangkul & Hashim, 1975; Haenlein, 1992; Park, 1994). In humans, Thomas *et al*. (2001) have reported that a medium-chain triglyceride (MCT) meal (containing 30% of fat calories) did not affect the triglyceride response to a fat meal. Alferez *et al*. (2001) found that the utilization of fat and weight gain was improved with a diet containing goat milk compared with one containing cow milk; levels of cholesterol were reduced while triglyceride, high-density lipoprotein (HDL), glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase values remained normal. The authors concluded that the consumption of goat milk reduces total cholesterol levels and the LDL fraction because of the higher percentage of MCTs (36% in goat milk vs. 21% in cow milk), which decreases the synthesis of endogenous cholesterol. According to McCullough (2003), the addition of MCTs to infant formula is controversial, because there is not enough evidence of an improved energy balance or weight gain; MCT side effects include distension, loose stools, and vomiting. However, infants fed MCT-containing formula absorbed significantly more calcium and magnesium than the control group (McCullough, 2003).

Milk from pasture-fed goats contains relatively higher amounts of CLA, which is best known for several beneficial and bioactive functions on human health, including anticarcinogenic, antiatherogenic, immune-stimulating and growth-promoting properties (Park, 2006). Gaullier *et al*. (2007) have also reported that supplementation of healthy overweight and obese adults with CLA decreases body fat mass in specific regions of the body and is well tolerated. Also, CLA supplementation for 7 ± 0.5 months decreased body fat mass in 6- to 10-year-old children who were overweight or obese, but did not improve plasma lipids or glucose and decreased HDLs more than in the placebo group (Racine *et al*., 2010). CLA is considered an important bioactive component in goat milk, which when enhanced by dietary changes also results in milk fat containing a lower proportion of saturated fatty acids and greater amounts of MUFAs (i.e., vaccenic acid) and PUFAs (Park *et al*., 2007).

Reynolds and Roche (2010) in their review report that synthetic and natural sources of CLA may have beneficial effects in inflammatory conditions, including colitis, atherosclerosis, metabolic syndrome, and rheumatoid arthritis. Most of the biological effects have been attributed to the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers. Evidence suggests that *cis*-9, *trans*-11 CLA is responsible for the anti-inflammatory effect attributed to CLA while *trans*-10, *cis*-12 CLA appears to be responsible for antiadipogenic effects.

BCFAs have also been studied in goat milk (Alonso *et al*., 1999). A comparatively high number of minor BCFAs has been found in goat milk, and the content of *trans*-C18:1 fatty acid is significantly lower in goat milk under average feeding regimes than in cow milk, thus reducing the risk of coronary heart disease (Haenlein, 2004).

23.4.2 The effects of milk proteins

Goat milk is widely used by people with digestive problems and sensitivities to cow milk products. The level of protein in goat milk is similar to that in cow milk but there are structural and immunological differences between the species, with goat milk having low (or no) α_{s1}-casein and higher α_{s2}-casein content and a greater proportion of β-casein, which makes it more similar to human milk in that respect (see Table 23.4). As goat milk has a relatively low α_{s1}-casein content, it is logical that children with high sensitivity to α_{s1}-casein in cow milk should tolerate goat milk quite well (Chandan *et al*., 1992). Moreover, goat milk has some special utilities in the treatment of allergy, particularly during childhood (Luke & Keith, 1992).

Rosenblum and Rosenblum (1952) have reported that gastrointestinal allergy in certain infants improved after administration and feeding of goat milk. Walker (1965) has also suggested, from clinical observation of infants and children, that individuals sensitive to cow ALA tolerated goat milk well. In over 25 years of conducting allergy clinics in three different hospitals, only 1 in 100 infants who were allergic to cow milk did not thrive on goat milk. Among 300 cases in which allergy to cow milk ALA was identified as the main cause of asthma and eczema, 270 subjects became symptom-free within 6 weeks after substituting goat milk for cow milk. During a 6-year period the investigation and treatment of several hundred more patients who were sensitive to cow milk appeared to confirm these conclusions (Walker, 1965). Later, after a number of scientific and clinical studies, Chandan *et al*. (1992) suggested that infants and children who were sensitive to cow milk-based products often thrive when a goat milk-based product is substituted. Furthermore, Soothill (1987) reported that children who were reactive or allergic to cow milk but not to goat milk also reacted to cow milk cheese but not to goat milk cheese. After long clinical studies in France with milk-allergic patients it was also concluded that substitution of cow milk with goat milk was tolerated with "undeniable" improvements (Sabbah *et al*., 1997).

Cow milk allergy is the most frequent allergy in the first years of life, and is considered a common disease with a prevalence of 2.5% in children during the first 3 years of life (Businco & Bellanti, 1993), occurring in 20–30% of infants under 3 months old (Lothe *et al*., 1982). The overall frequency in Scandinavia is 7–8% (Host *et al*., 1988), even as high as 20% in some areas (Nestle, 1987), while in Italy it occurs in 3% of children under 2 years of age (Bevilacqua *et al*., 2000). Treatment with goat milk resolved between 30 and 40% of the problem cases, and in one particular study 49 of 55 treated children benefited from treatment with goat milk (Haenlein, 2004). Brenneman (1978) also reported that in his experiment, about 40% of allergic patients sensitive to cow milk proteins were able to tolerate goat milk proteins. Those patients were sensitive to cow ALA, which is species specific. Cow milk allergy may also be due to BLG (Heyman & Desjeux, 1992).

Allergy toward a given food may develop, particularly in the first year of life, for any food that is different from the nursing mother's milk (Noimark & Cox, 2008). Therefore, it is not surprising that allergies specific to goat milk have been identified (Ah-Leung *et al*., 2006). In the absence of maternal milk, allergic subjects need an alternative protein source, which is usually based on hydrolyzed cow milk proteins (caseins or whey proteins) or soybean-based formula. Milk from various mammalian species (horse, donkey, and goat) has been suggested as a positive alternative for cow milk allergy (Park & Haenlein, 2006). However, symptoms of allergy to goat milk appeared at a much later age than those to cow milk (Ah-Leung *et al*., 2006), which may benefit younger infants who are dependent on milk as their main source of nutrients. In addition, goat milk allergy-related symptoms may develop in individuals who have already developed an allergy to cow milk (Bellioni-Businco *et al*., 1999). However, an average of five times more goat milk than cow milk was required to trigger an adverse reaction, lending some support for a difference in the allergenic potential of goat and cow milk (Bellioni-Businco *et al*., 1999). Administering clinical skin-prick tests on 21 adults and 13 infant patients with suspected cow milk allergies, ALA caused the most positive skin reactions (Haenlein, 2004). Of the 13 infants, 10 showed positive reactions, while only 5 of 21 adults reacted (Kaiser, 1990). Of these five adults, only one had a weak IgG titer against ALA.

Bevilacqua *et al*. (2001) has suggested that the reduced allergenicity of goat milk might be directly related to its lower levels of α_{s1}-casein or to a modified ratio of BLG to α_{s1}-casein. Resistance to digestion is a key determinant in the allergenicity of protein (Astwood *et al*., 1996). Studies have reported that goat milk forms a finer curd than cow milk upon acidification at similar levels to those found in the stomach (Haenlein, 1992). The low levels of α_{s1}-casein are considered a key reason for goat milk forming a softer curd of higher digestibility (Clark & Sherbon, 2000). The more efficient digestion of BLG provides a possible explanation for the reduced allergenic properties of goat milk (Bevilacqua *et al*., 2001). Since the level of α_{s1}-casein varies among breeds and across seasons, and not all goat milks contain a low level of α_{s1}-casein, the level of allergic response may vary (Prosser, 2005; Moioli *et al*. 1998, 2007).

Ten genetic allergic variants (A, B_1, B_2, B_3, C, D, E, F, G, and O) of the protein α_{s1}-casein have been identified in goat milk that affect α_{s1}-casein synthesis and which are

Table 23.6. α_{s1}-Casein genetic variant frequencies (%) found in different dairy goat breeds.

	All breeds (total)	A	L	N	O	S	T	C
A	9.7	2.7	20.6	25.0	0.0	9.1	5.6	13.6
B_2	4.8	6.8	2.9	0.0	0.0	9.1	5.6	0.0
B_3	2.2	1.4	2.9	8.3	0.0	4.6	0.0	0.0
C	2.7	1.4	2.9	8.3	0.0	0.0	0.0	9.1
D	4.8	8.1	2.9	8.3	0.0	0.0	0.0	4.6
E	18.3	20.3	17.6	0.0	50.0	31.9	5.6	13.6
F	52.7	54.1	50.0	41.7	50.0	45.5	83.3	41.0
O	4.8	5.4	0.0	8.3	0.0	0.0	0.0	18.2
N	93	37	17	6	2	11	9	11

A, Alpine; L, La Mancha; N, Nubian; O, Oberhasli; S, Saanen; T, Toggenburg; C, crossbred does include four N×A; one L×A; three S×A; one S×O; one N×O; and one A×L×N×S. Genetic variants B_1 and B_2 were not separable by the method used in this research. For the purposes of this table, samples that could have included B_1 or B_2 are called B_2. Variant G was not determined.

Source: reproduced from Clark & Sherbon (2000), with permission of Elsevier.

correlated with total milk nitrogen, casein, and fat content (Clark & Sherbon, 2000). The "high type" variants (A, B, C) have been associated with higher amounts of α_{s1}-casein and are normally high in caprine species, while the other alleles are defective mutants. The "low type" variants (D and F) are associated with low amounts of α_{s1}-casein in goat milk, whereas milk which is homozygous for the "null type" variant O is lacking α_{s1}-casein (Grosclaude *et al*., 1987). Such α_{s1}-casein genetic allelic variant frequencies found in different goat breeds have been reported by Clark and Sherbon (2000) and Moatsou *et al*. (2006, 2008). The α_{s1}-casein genetic variant frequencies of different breeds of dairy goats are presented in Table 23.6.

It is important to evaluate the gene frequency of α_{s1}-casein alleles in different dairy goat breeds and to determine the effect of the α_{s1}-casein genotype as a criterion in selection programs, since quantitative alleles determine differences in nutritional and technological properties of milk through the modification of the ratios among protein fractions. Thus, through genotypic information at the α_{s1}-casein locus, the frequency of the favorable alleles can be increased and/or the frequency of the unfavorable ones can be decreased. If the primary objective, for instance, is cheesemaking, selection for "high type" α_{s1}-casein genetic variants is recommended. In contrast, if the primary objective is allergy treatment, then the "null type" variant is recommended.

Moatsou *et al*. (2008) have found that in two different groups of goats of different breeds (indigenous Greek and International), there were two levels of α_{s2}-casein content: low and high. There were three genotypes assigned to variant A (or B), C, and F in the samples of indigenous Greek goat breeds that were clearly separated using reversed-phase high-performance liquid chromatography coupled to electrospray ionization mass spectrometry. The milk samples from the international breeds were classified into three groups. In this case, three α_{s2}-casein variants, A, B, and C, were observed, the most abundant being variant A followed by variant C. Variant F was not observed. Two variants of α_{s2}-casein, A and B, have also been described, differing in replacement of amino acids Asn49 and Lys200 by Asp49 and Asn200 (Park *et al*., 2007).

According to the literature, variant A is generally predominant in the milk of most goat breeds. The frequency of goat α_{s2}-casein A, B and C in French dairy breeds, and Alpine and Saanen breeds has been found to be 0.85, 0.04, and 0.11 respectively (Bouniol *et al*., 1994). Erhardt *et al*. (2002) reported that variant A is predominant in German and Italian breeds (from 0.662 to 0.922), while variant B occurs at frequencies lower than 10% or at zero frequencies, and variant C occurs with higher frequency in Italian breeds than in German. According to the findings of Sacchi *et al*. (2005), variant F is also abundant, as is variant A in Italian goat breeds followed by variant C, whereas variants B and D and the null variant are the most rare.

Mora-Gutierrez *et al*. (2007) suggested that caprine Monterey Jack cheese characterized by a high content of α_{s2}-casein and β-casein peptides, unfortified or fortified with milk calcium, may be more protective against bone fragility than bovine Monterey Jack cheese, most likely by enhancing calcium and magnesium absorption in the gut.

Other extensive clinical studies by Reinert and Fabre (1997), Fabre (1997), and Grzesiak (1997), in children allergic to cow milk, showed that after treatment with goat milk, positive results were produced in 93% of the children; it was recommended that goat milk is a valuable aid in child nutrition because of its lower allergenicity and better digestibility than cow milk, which helps babies to settle and maintain a healthy gastrointestinal system.

Thus, the main reason for the hypoallergenic value of goat milk, compared with cow milk, is the difference between their protein structures (Imafidon *et al*., 1991), which results in higher digestibility. Further, Almaas *et al*. (2006) showed that goat milk proteins were digested by human gastric and duodenal enzymes faster than cow milk proteins.

Prosser *et al*. (2004) have shown that regular consumption of goat milk can reduce gut leakiness, inflammation, and damage to villi. When Infante-Pina *et al*. (2003) tested the

tolerance to goat milk of 12 children with allergy to cow milk, using a range of immunological tests and oral challenge, they found that 25% of the children were negative for the immunological tests and showed tolerance to the oral challenge with goat milk.

Evaporated goat milk or goat milk powder has been recommended for infant formula by Taitz and Armitage (1984) and Coveney and Darnton-Hill (1985), since heat applied to manufacturing processes reduces allergic reactions (Perlman, 1977). Some goat milk proteins have immunological cross-reactivity with cow milk proteins, but infants suffering from gastrointestinal allergy and chronic enteropathy against cow milk were reportedly cured by goat milk therapy (Park, 1994). Spuergin *et al*. (1997) suggested that sheep milk and goat milk harbor an allergic potential and are not suitable for nutrition in milk-allergic patients. Calvani and Alessandri (1998) and Orlando and Breton-Bouveyron (2000) have also suggested that goat and sheep milk allergy can be present without cow milk allergy.

In an attempt to reduce allergenicity, Ramunno *et al*. (2001) reported a mutation that revealed a gene associated with an undetectable amount of α_{s2}-casein in goat milk, which could reduce allergenicity. The occurrence of alleles associated with null or faint content of the different caseins might be exploited for the production of milk with particular nutritional qualities (i.e., hypoallergenic properties for those subjects monosensitized to this protein fraction) (Moioli *et al*., 1998, 2007).

The allergenicity of milk should be assessed by appropriate clinical studies before selecting animals within a certain group of *CSN1S1* genotypes. Ballabio *et al*. (2011) concluded that the use of goat milk for infant formulae might be suggested only in well-selected cases and after appropriate nutritional modifications. In fact, although the use of goat milk in hypoallergic formulae is not generally recommended, positive results could be achieved in the preparation of modified formulae suitable for defined groups of allergic patients (Ballabio *et al*., 2011).

23.4.3 The effects of milk bioactive peptides

Milk proteins comprise peptides with particular biological activities that also cause symptoms of allergies. Biologically active peptides are liberated by digestion or proteolysis of caseins and whey proteins and possess very important biological functions, including antimicrobial, antihypertensive, antioxidative, anticytotoxic, immunomodulatory, opioid, and mineral-carrying activities, with actions on the gastrointestinal, cardiovascular, endocrine, immune, and nervous systems (Seppo *et al*., 2003; Korhonen & Pihlanto, 2007). Chessa *et al*. (2009) found that of the bioactive peptides which goat milk shares with bovine milk, nine show opioid activity, five are mineral carriers, 23 show hypotensive activity, three are antithrombotic, and six are immunomodulators. They also concluded that attempts to evaluate local breeds for their casein variation, particularly with regard to human nutrition and health, should take into account the different genetic variants of goat milk bioactive peptides. Bioactive peptides can also be released from milk proteins during milk fermentation and cheese maturation, which thus enriches the dairy products (Gobbetti *et al*., 2002).

Like human milk, goat milk naturally contains bioactive peptides such as nucleotides, polyamines, sialic acid, free amino acids, and growth factors that promote optimum development. The antimicrobial activity of milk is mainly attributed to immunoglobulins and to non-immune proteins such as lactoferrin, lactoperoxidase, and lysozyme (Atanasova & Ivanova, 2010). The total antibacterial effect in milk is greater than the sum of the individual contributions of immunoglobulins and non-immunoglobulin defense proteins. Antibacterial peptides have been found to be active against a broad range of pathogenic microorganisms, such as *Escherichia*, *Helicobacter*, *Listeria*, *Salmonella* and *Staphylococcus*, yeasts and filamentous fungi (Atanasova & Ivanova, 2010).

23.4.3.1 Angiotensin I-converting enzyme

Among the known bioactive peptides, Geerlings *et al*. (2006) have identified and characterized new inhibitory peptides of angiotensin I-converting enzyme (ACE) from goat milk, and these have beneficial effects in the treatment of hypertension after long-term intake of a goat milk hydrolysate-supplemented diet. ACE inhibitory peptides derived from the milk caseins and whey proteins of cow, goat, and sheep milk have different activities (Park *et al*., 2007), and goat milk proteins are regarded as an important source of ACE inhibitory peptides (Lee *et al*., 2005; Quirós *et al*., 2005).

23.4.3.2 Nucleotides

Nucleotides are found in human and other species' milk at varying levels. Animal and human *in vitro* studies have shown that nucleotides play an important role in stimulating gastrointestinal growth, maturation, and recovery (Carver & Walker, 1995) and contribute to immune enhancement (Aggett *et al*., 2003). Goat milk contains a complex array of nucleotides that facilitate immune system maturation in offspring fed on milk; hence nucleotides are part of infant formulae (Schaller *et al*., 2007). Prosser *et al*. (2008) showed that infant formulae made from goat milk have the same levels of nucleotides as human milk and do not need additional nucleotides. Iron bioavailability is higher in goat milk than in cow milk due to the higher nucleotide content of goat milk, and this contributes to better absorption of iron in the gut (Park *et al*., 1986).

23.4.3.3 Polyamines

Polyamines (i.e., spermidine, spermine, putrescine) are essential for cell growth and differentiation. It has been suggested that they may play a role in preventing or reducing sensitization to food allergens (Dandrifosse *et al*., 2000). The total polyamine concentration naturally present in goat milk has been found to be higher than in cow milk and similar to human milk (Prosser, 2005).

23.4.3.4 Sialic acid

Sialic acid is found in high concentrations in brain gangliosides, which are thought to be important structural and functional elements of the human brain. Since infants may not be able to sufficiently or efficiently synthesize sialic acid because of the immaturity of their liver (Wang *et al*., 2001), supplementary sialic acid from the diet, particularly milk, may be important in providing sufficient amounts for optimal brain development in infants. The sialic content of human milk is approximately 30 mg/dL and that of goat milk about 8 mg/dL (Wang *et al*., 2001).

23.4.3.5 Taurine

Of the free amino acids, taurine has been found to play a role in relation to membrane stabilization, bile salt formation, antioxidation, calcium homeostasis, growth modulation and osmoregulation, with some potential immunoregulatory properties (Redmond *et al*., 1998). Goat milk contains much taurine and contributes to the treatment of diabetics (Anaeto *et al*., 2010). The infant has a limited capacity to synthesize taurine and because of this the fetus accumulates taurine over the full gestation period.

23.4.3.6 Growth factors

Human milk contains a range of growth factors, such as insulin-like growth factor (IGF)-1, IGF-2, IGF-binding protein, epidermal growth factor, transforming growth factor (TGF)-α, and TGF-β, which are physiologically important molecules produced by the body and can initiate cellular growth and the expression of many differentiated functions (Penttila *et al*., 2003; Ogawa *et al*., 2004). Some of these growth factors have been confirmed present in goat milk but their physiological role should be further investigated.

Because of its capacity to produce nitric oxide, goat milk may exert a cardioprotective and antiatherogenic effect in humans. Moreover, induction of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 and of anti-inflammatory cytokines such as IL-10 suggests that goat milk has the ability to maintain homeostasis in the immunocompromised host (e.g., elderly people) (Martemucci *et al*., 2010).

23.4.4 The effects of milk oligosaccharides

Milk oligosaccharides have considerable antigenic properties and are valuable in promoting the growth of intestinal flora in the newborn (Park & Haenlein, 2007). Goat milk may be of great nutritional interest, especially regarding the highly bioactive oligosaccharide fraction (Boehm & Stahl, 2007). In support of this observation, it has been recently demonstrated that goat milk oligosaccharides possess anti-inflammatory properties in a rodent model of hapten-induced colitis (Daddaoua *et al*., 2006; Lara-Villoslada *et al*., 2006; Martinez-Férez *et al*., 2006). Gopal and Gill (2000) have also reported the contribution of oligosaccharides in neonatal brain development.

23.4.5 The effects of milk minerals and vitamins

The high levels of calcium in milk play an important role in the development, strength, and density of bones in children, and in the prevention of osteoporosis in elderly people. Calcium has also been shown to be beneficial in reducing cholesterol absorption, and in controlling body weight and blood pressure (Park, 2009).

The influence of goat and cow milk on the nutritive utilization of iron has been studied by López-Aliaga *et al*. (2010), who found that goat milk has a beneficial effect on the metabolism of calcium and iron, which minimizes any interaction between the two minerals.

The adequacy of goat milk to meet human requirements for minerals and vitamins has not been much studied. However, most basic minerals are present in much higher concentrations in goat and cow milk than in human milk and they present a high solute load when fed undiluted to infants. On the other hand, infants fed exclusively on goat milk are at risk of developing megaloblastic anemia, unless the goat milk is fortified with folate (O'Connor, 1992). Besides being low in folate, goat milk is also low in vitamin B_{12} content compared with cow milk, but goat milk has a much higher content of vitamin D, thiamin, riboflavin, niacin, pantothenic acid, vitamin B_6, and biotin than human milk (Park, 2006). In determining the level of supplemental folate, the effect of heat treating the milk must also be considered, as infants fed heat-treated cow milk have developed signs of folate deficiency (Ek & Magnus, 1980). Other studies have confirmed that thermal processing and certain pasteurization procedures result in degradation and significant negative impacts on folate content in milk (Ristow *et al*., 1982; Picciano, 1985).

23.4.6 Goat milk products

Goat milk is widely used to produce different cheeses and yogurt. Moreover, other products such as cream, butter, ice cream, candy, whey proteins, condensed and dried milk

products, beverages, and a number of indigenous milk products are made from goat milk worldwide and have been described by Pandya and Ghodke (2007), Park and Haenlein (2007), and Ribeiro and Ribeiro (2010).

23.4.6.1 Fermented milk, yogurt

The functional value of goat milk may be further exploited through fermentation by selected microorganisms possessing specific features (Minervini *et al*., 2009). The "functional foods" group contains selected bacteria such as *Lactobacillus acidophilus* or *Bifidobacterium* spp., which provide several prophylactic and therapeutic benefits (Ishida *et al*., 2005). The viable lactic acid bacteria in fermented milk products have been associated with increased lactose tolerance, a well-balanced intestinal microflora, antimicrobial activity, stimulation of the immune system, and antitumoral, anticholesterolemic, and antioxidative properties in human subjects (Kullisaar *et al*., 2003; Sanna *et al*., 2005; Songiseep *et al*., 2005). Additionally, fermented goat milk loses its characteristic "goaty" taste, which is unacceptable for many consumers (Haenlein, 2004).

Fermented dairy products have long been thought to confer special health benefits. The cultures in fermented dairy products, especially "Bulgarian bacillus" (*Lactobacillus delbrueckii* subsp. *bulgaricus*), help to prevent intestinal putrefaction, thereby prolonging life. Yogurt, a fermented dairy product, is a nutritious food that can deliver multiple health benefits to consumers (Boycheva *et al*., 2011). Research into the health benefits of yogurt have focused on traditional yogurt cultures and adjunct cultures that are often added for their health benefits. Some of the health benefits that have been postulated for yogurt and probiotics include lower serum cholesterol, immune system stimulation, reduced risk of colon cancer, reduced lactose intolerance, increased calcium absorption, improved digestive regularity, and normalization of intestinal microflora (Pannell & Schoenfuss, 2007).

Scientific investigation of the benefits of ingesting yogurt cultures has focused on the benefit to lactose-intolerant individuals. During fermentation, the lactose content of milk is reduced by up to 30% (Adolfsson *et al*., 2004). Approximately 80% of adults in the world have lost their ability to split the β-glycosidic bond in lactose. The reduction of lactose by fermentation and the active enzymes released from yogurt cultures when cells burst have been shown to improve the digestibility of the milk and yogurt for lactose-intolerant individuals. In addition to the required yogurt cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), probiotic cultures (*Lactobacillus acidophilus*, *Bifidobacterium* spp.) are increasingly being added to yogurt (Donkor *et al*., 2007). Adolfsson *et al*. (2004) have summarized the studies of yogurt and probiotic cultures and their effects on health.

23.4.6.2 Cheeses

Goat cheeses have been described in many books (Mills, 1988; Anifantakis, 1991a; Harbutt & Denny, 2002; Kaufelt & Thorpe, 2006). From among more than 300 of the world's best-known cheeses, 60 different pure goat milk cheeses and 18 mixed milk cheeses (goat and cow or sheep milk) are available for sale in one of New York City's premier cheese shops (Kaufelt & Thorpe, 2006). Probably the best-known goat cheeses come from France, where one popular cheese, Bouche du Poitou, had an annual production in 2001 of 4268 tons accounting for 6.6% of total French goat cheese production (Rubino *et al*., 2004). Also during the past 30 years there has been a rebirth and phenomenal growth of US dairy goat farming, with artisanal goat cheesemaking supported and inspired by popular cheese contests at the annual conventions of the American Cheese Society and the Wisconsin Cheese Makers Association. At the 2012 World Championship in Wisconsin, 2504 cheeses were entered in 82 classes, including six classes for goat cheeses: soft, flavored soft, surface mold ripened, semi-soft, flavored semi-soft, hard, and goat butter (Archwamety, 2012). The most famous Greek cheese produced mostly from goat and/or sheep milk is Feta, but its firmness, duration of ripening, microbiological characteristics and sensory properties vary, depending on locality and traditions (Anifantakis, 1991b). It must be noted that according to Greek tradition, goats and sheep are always herded together, as also is the case in many Mediterranean and Middle Eastern countries; therefore, their milk is always mixed, which is in contrast to typical indoor goat farming, for example in France, where there is no admixture of sheep. The free fatty acids and free amino acids give flavor to Feta cheese, while total microflora, lactic acid, and proteolytic bacteria reach high values at 45 days. In addition to Feta, many types of artisanal cheeses are produced in Greece from raw or heat-treated goat milk and/or from whey. Some of these cheeses are listed below:

- Mantzios: semi-hard cheese.
- Telemes: soft cheese from sheep and goat milk inoculated with *Streptococcus lactis* and *L. bulgaricus* in a ratio of 1 : 3 and 0.5% lactic acid cultures.
- Kopanisti: soft cheese from sheep and goat milk after intense proteolysis and lipolysis take place, giving the characteristic unique flavor to this cheese.
- Kefalotyri: hard cheese from sheep and goat milk with lactic acid cultures.
- Graviera: hard cheese from sheep and goat milk inoculated with *S. lactis* 1% and *Streptococcus cremoris* and *Lactobacillus helveticus* 0.1%.

- Manouri: semi-soft cheese from whey of goat and/or sheep milk.
- Mizithra: fresh or hard cheese from whey of goat and/or sheep milk after Feta production.
- Anthotiros: fresh or dried cheese from whey of goat and/or sheep milk after production of Kefalotyri and Graviera.

The natural lactic microflora of raw goat milk used for cheese production may play an important role in the manufacture of cheese. High counts of anaerobic bacteria, lactic acid bacteria, psychrotrophs, and proteolytic and lipolytic bacteria were recorded throughout ripening of white-brined cheese by Litopoulou-Tzanetakis and Tzanetakis (1992). The main species of lactic acid bacteria isolated from raw goat milk were *Enterococcus durans*, *Lactobacillus plantarum* and *Leuconostoc paramesenteroides*.

The large number of varieties of cheese made from goat milk has resulted in great diversity in the nature of the products. Paneer is an important indigenous Indian cottage-type cheese, while Gjetost cheese of Norway is made from goat milk whey, where caramelized lactose in concentrated whey is combined with fat and whey proteins (Ribeiro & Ribeiro, 2010). Ricotta is another well-known cheese made in Italy from whey of goat and/or sheep milk. A traditional fresh cheese called Labaneh is very popular in Middle Eastern countries (Rubino *et al*., 2004). It starts as drained whole yogurt from goat, sheep, or cow milk, but goat Labaneh (35 kg from 100 L milk) is sold at a 30% higher price than sheep or cow milk Labaneh. Bedouins dry the Labaneh in the sun after heavy salting to create desiccated cheese that lasts for several months.

23.4.6.3 Powder and condensed milk

As stated before, goat milk powder or evaporated goat milk has been recommended for use as infant formula (Taitz & Armitage, 1984; Coveney & Darnton-Hill, 1985), since heat in the manufacturing processes reduces allergenicity (Perlman, 1977). Most infant formulae use vegetable oils in place of milk fat to provide an overall fatty acid profile similar to that of human milk. Vegetable oils have 5–20% saturated fatty acids in the *sn*-2 position of triglycerides unless they are modified by interesterification, which is increasingly used for the fat in infant formulae to raise the level of saturated fatty acids in the *sn*-2 position to 40–60%. Mixing goat milk fat with vegetable oils produced a formula with a profile of essential fatty acids and a ratio of linoleic to α-linolenic fatty acids within the required interval of 5 to 15 : 1 recommended for infant formula (Prosser *et al*., 2010).

Powdered products include powdered whole milk, skim milk, whey, and infant foods (Park, 2005). According to Pandya and Ghodke (2007), literature on the manufacture of powdered goat milk is limited, possibly because of the unavailability of large quantities of goat milk from low-production farms. Kruger *et al*. (2008) demonstrated that goat milk powder, when used as a source of proteins and minerals supplemented with prebiotics and probiotics, improved mineral absorption and retention in human digestion. Evaporated (condensed) goat milk (sweetened or unsweetened) is usually made under reduced pressure, primarily to allow boiling at a lower temperature to prevent heat damage (Ribeiro & Ribeiro, 2010). The value of these products in human nutrition has not been studied as extensively as it has for cheeses and fermentation products like yogurt. In 1934 John Meyenberg started commercial production of evaporated milk in California for distribution in pharmacies targeting the medical profession (Jackson, 1992). In the 1960s, goat milk powder and UHT goat milk were added by the Jackson-Mitchell Meyenberg Company in California to the growing health food market. Whole goat milk powder commercial production was started in Brazil in 1994 (Rubino *et al*., 2004), and powder is also produced in New Zealand for the infant formula market.

23.4.6.4 Butter

Commercial production of salted and unsalted goat butter has an undeservedly limited distribution, but European-style goat butter produced by the Jackson-Mitchell Meyenberg Company in California and whey cream goat butter produced by the Mt. Sterling Creamery Cooperative in Wisconsin have received international awards (Archwamety, 2012) and are marketed by mail order. Stakovutiro, also called butter oil, is made in Greece on the island of Crete from sour cream of goat and/or sheep milk with some flour added and after boiling collected in bottles by shepherds on farms. Research reports on goat butter are hard to find.

23.4.6.5 Other goat milk products

Milk proteins are the main source of a range of biologically active peptides. Proteolysis may produce these biogenic peptides during food processing and during gastrointestinal transit. Enzymes from different sources, including microbial enzymes, generate bioactive peptides during milk fermentation and cheese ripening, thereby enriching dairy products (Gobbetti *et al*., 2002). In a number of recent studies on native Spanish cheeses from goat milk, the composition, microbiology, biochemistry, and changes during ripening have been comprehensively described (Fontecha *et al*., 2006; Calvo *et al*., 2007). Such studies have reported the antimicrobial activity of lactic acid bacteria isolated from these cheeses (Casla *et al*., 1996; Herrero & Requena, 2006). *Lactobacillus casei* is also the predominant probiotic strain in the Sicilian cheese Provola

dei Nebrodi (Cronin *et al*., 2007). An extensive survey with descriptions of goat products worldwide, including gelato goat milk ice cream from Italy, Spain, and Australia, so-called "dolce" sweets from goat milk in South America and Mexico, cosmetic soap products and lotions from goat milk cream, goat milk powder, and about 140 goat cheeses, has been published by the Small Ruminant Research Institute in Potenza, Italy (Rubino *et al*., 2004). In 2002, estimated production of 25 000 tons of Cajeta caramel sweets and 30 000 tons of Sevillana candy besides 79 000 tons of cheese from 1.1 million goats producing 389 000 tons of milk was reported for Mexico (Galina & Haenlein, 2004). The economic value of the goat milk industry has been estimated for Australia, for example, to be 2–3 million liters per year with a farm-gate value of US$1.4 million, and about 20 processors produce about 165 tons of cheese valued at US$1.9 million (Rubino *et al*., 2004).

23.5 CONCLUSIONS

The chemical composition of goat milk, even though it varies according to genotype, stage of lactation, parity, season and feeding, is superior to cow milk and is more similar to breast milk. It is often considered a functional food for infants, children, and elderly people because it contains a variety of different bioactive components like caseins, whey proteins, MCFAs, oligosaccharides, and minerals. Goat milk, apart from its ability to reduce cow milk allergenicity, plays an important biological role in the gastrointestinal, cardiovascular, endocrine, immune and nervous systems, including antimicrobial, antihypertensive, antioxidative, anticytotoxic, immunoregulatory, opioid, and mineral-carrying activities. Thus, because of goat milk's nutritional value, digestibility, therapeutic, and dietary characteristics, it is becoming more relevant for the human diet, particularly for certain sectors of the population with particular needs.

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24
Buffalo Milk

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24.1 INTRODUCTION

Buffalo milk has an important place in the livestock and dairy sector of India and Pakistan (two major buffalo milk-producing countries with 92% of the world's total buffalo milk production; see Table 24.2). Both these countries are among the top milk-producing countries of the world. In India and Pakistan, buffalo milk's share of national milk production compared with other dairy species is highest, at 53% and 63%, respectively (FAOSTAT, 2010). Buffalo milk is a whiter fluid than other mammalian milk (due to higher concentrations of caseins and absence of carotenes) and consists of globules of fat and casein micelles in suspension, and whey proteins, milk sugar and inorganic salts in solution, as in milk from other mammals. All these major constituents are in higher concentrations than in human, cow, goat and camel milk. Thus, this milk can be considered a good energy and nutrition source not only for buffalo calves but also for humans. Its nutritional importance is proven by the quick and easy growth of the calf that occurs because of the higher percentages of butterfat and milk solids in the dam's milk.

24.1.1 Buffalo populations and breeds

Asia is the home of water buffaloes, with more than 85% of buffalo in only three countries of south and east Asia: India, Pakistan and China (Table 24.1). According to FAOSTAT (2010), there are 194.2 million head of buffalo in the world, the distribution of which can be seen in Table 24.1. The buffalo is a multipurpose animal and has been an integral part of livestock agriculture in Asia for over 5000 years, producing draught power, milk, meat and hides (Amarjit & Toshihiko, 2003). The main buffalo dairy breeds include Murrah, Nili-Ravi, Surti, Kundi, Banni, Mehsana, Azeri, Toda, Mediterranean, Pandharpuri, Albino, Bhadawari, Jaffarabadi, Nagpuri, Sambalpur, Kalahandi, Kanara, Chilika, Tarai, Kujang, Paralakhemundi and Manda. Breed characteristics for the majority of these breeds, their genetic, reproductive and milk production traits along with nutrition and energy requirements have been reviewed by Pandya and Khan (2006). Some buffalo dairy breeds can be seen in Fig. 24.1 (see also Plate 24.1). Buffalo males may be used for traction, and all animals are eventually used for meat. Buffaloes in other parts of the world like Egypt, Eastern Europe (Bulgaria, Romania, the former Yugoslavia and the former Soviet Union) and Italy are used for milk production, and there are also herds used principally for this purpose in Iran, Iraq and Turkey (Ligda, 1998).

24.1.2 Buffalo milk production and consumption

Buffalo milk is the second most produced milk in the world after cow milk (92.5 and 599.6 billion litres contributing 12.8% and 83.2%, respectively) (FAOSTAT, 2010). Major buffalo milk-producing countries of the world, their production rates and percentage contributions to world buffalo milk production are given in Table 24.2. The buffalo milk industry is an unorganised sector in Asia and Africa, whereas in Europe (Italy) it is well organised. A great part of this milk production goes unrecorded. An individual buffalo producing 3000 L per lactation was considered a record about four decades ago, but now animals can produce up to 5000 L or even more (Jainudeen, 2002). The daily yield of lactating

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Table 24.1. World buffalo population.

Country	Buffalo head (millions)	Percentage
India	111.3	57.3
Pakistan	30.8	15.9
China	23.6	12.2
Nepal	4.8	2.5
Egypt	4.0	2.1
Philippines	3.3	1.7
Myanmar	3.0	1.5
Vietnam	2.9	1.5
Indonesia	2.0	1.0
Thailand	1.6	0.8
Bangladesh	1.3	0.7
Brazil	1.2	0.6
Timor-Leste	1.1	0.6
Cambodia	0.7	0.4
Islamic Republic of Iran	0.7	0.4
Sri Lanka	0.4	0.2
Italy	0.3	0.2
Iraq	0.3	0.2
Malaysia	0.1	0.1
Total head	194.2	100.0

Source: based on data from FAOSTAT (2010).

buffaloes in Asia may be as low as 2.5 kg (small family farms) and as high as 20.0 kg (well-managed progressive farms). With selective breeding, improved management and the establishment of more dairy herds, buffalo milk yields are steadily increasing worldwide at a rate of more than 3% per year (International Dairy Federation, 2008). The potential for increased milk production therefore exists for this species. Dairy buffaloes are commonly known as water buffaloes and their production is very much associated with environmental conditions. Their milk yield decreases with high ambient temperature. The daily milk yield can be improved by splashing them with water twice daily before milking (Sinha & Minett, 1947), which reduces body temperature. Providing wet screens around the shed can also improve feed consumption and milk yield. Buffalo milk is used in much the same way as cow milk. In major buffalo milk-producing countries (India, Pakistan and Egypt), it is drunk either raw at farm level or after severe boiling in the villages or urban areas at domestic commercial distribution levels. The rich and precious buffalo milk may be converted to cream, butter, yoghurt and many cheeses (Michelizzi *et al*., 2010). There is growing interest in the nutritional aspects of buffalo husbandry around the world (Spanghero & Susmel, 1996).

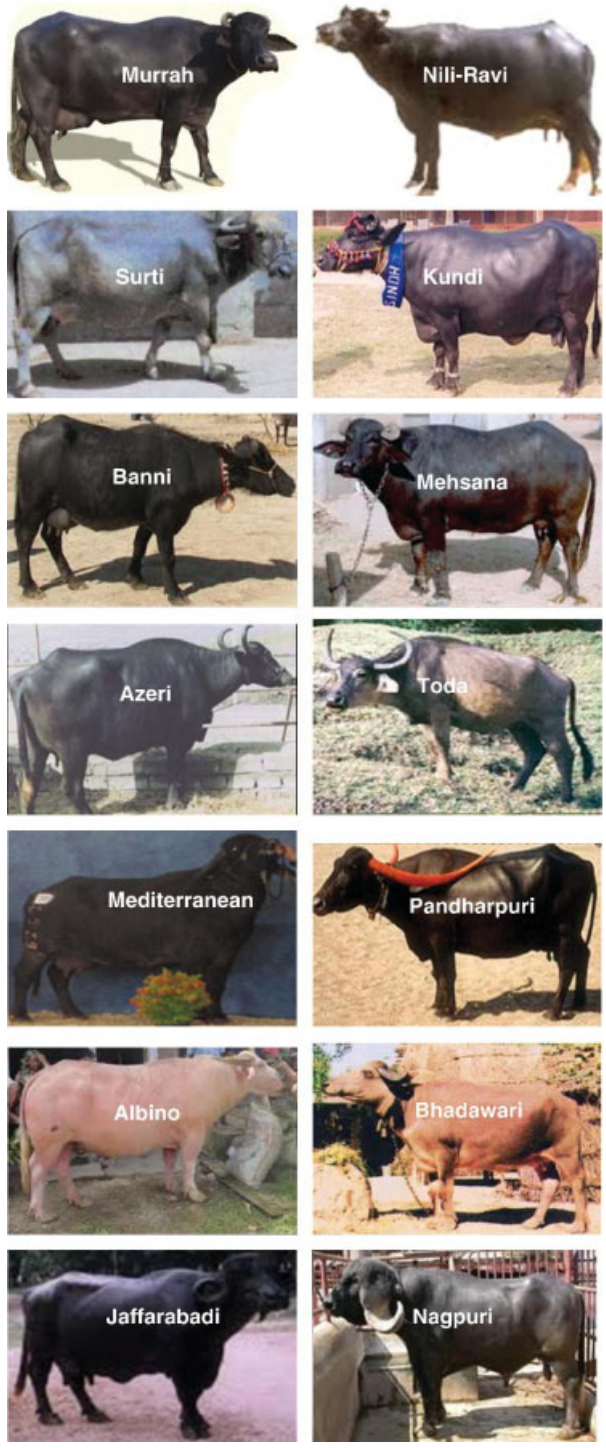


Figure 24.1. Major dairy buffalo breeds. For a colour version of this figure, see Plate 24.1.

Table 24.2. Worldwide buffalo milk production and its distribution by country.

Country	Production (billion litres)	Percentage
India	62.40	67.4
Pakistan	22.28	24.1
China	3.10	3.4
Egypt	2.73	3.0
Nepal	1.07	1.2
Islamic Republic of Iran	0.28	0.3
Myanmar	0.25	0.3
Italy	0.21	0.2
Sri Lanka	0.05	0.05
Bangladesh	0.036	0.04
Turkey	0.036	0.04
Vietnam	0.035	0.04
Iraq	0.024	0.03
Malaysia	0.011	0.01
Total milk production	92.51	100.0

Source: based on data from FAOSTAT (2010).

24.1.3 Socioeconomic importance of buffaloes

People from developed countries have a picture of buffalo as a wild animal and the majority do not even know about the existence of buffalo milk. These animals play a pivotal role in the economy of countries where buffalo milk is produced by providing essential items to the human diet in the form of milk and meat and a significant share of the gross domestic product. The economic potential gained by the extensive and intensive exploitation of buffalo milk production is satisfying much of the nutritive requirements of the population (1.4 billion people in India and Pakistan, i.e. more than 20% of the world's population) and this is increasing the interest in buffalo breeders. These animals are an important part in the lives of small farmers, while larger buffalo herds are reared by rural communities. In Pakistan, for example, about 35 million rural people are engaged in buffalo raising, deriving 30–40% of their income from it (Bilal & Ahmad, 2004). A dairy buffalo is generally looked upon as a prestigious possession, as the number of buffaloes kept by farmers determines their wealth and status in society. Owing to the versatility of buffaloes, they are rightly called 'black gold' by farmers.

The nutritional physiology of buffaloes is similar to that of cows (Pandya & Khan, 2006); however, buffaloes are apparently more efficient in converting highly fibrous low-grade feeds like paddy straw and crop residues (i.e. poor-quality roughage) into high-quality nutritional milk. Buffaloes have a 5% higher digestibility for crude fibre than cows, and are 4–5% more efficient when using metabolic energy for milk production (Mudgal, 1988). Although they eat poor-quality roughage, buffaloes grow faster than cattle because of their better digestibility (Sebastian *et al*., 1970). Coefficients of apparent digestibility of dry matter, neutral detergent fibre and crude protein in the diet and growth rate were found to be higher for buffaloes than cows (Nha *et al*., 2008). The cost of fattening per kilogram body weight is therefore much lower for buffaloes than for cattle (Chantalakhana, 2001). Otherwise, these feed by-products and wastes would lead to an increase in environmental pollution, which is a most serious issue at present in the global dairy sector. So, buffaloes have the ability to subsist on a low-quality, high-roughage diet. They are resistant to most internal and external parasites that affect cattle and are capable of maintaining better quality milk and maintaining their health.

24.1.4 Buffalo milk commercial products

Currently, the buffalo milk market is mainly based on traditional dairy products and dealt with in informal ways in all buffalo milk-producing countries. The higher milk solids content of buffalo milk not only makes it ideal for processing into superb dairy products but also contributes to significant energy savings during the process. There is a long list of buffalo milk-derived products such as sweetmeats which have been prepared in Asian subcontinent (now India, Bangladesh and Pakistan) households since time immemorial, including khoa, dahi, paneer, kheer, rabri, sheer-khorma, malai, kulfi, ghee, chhana, shrikhand, and several milk confections prepared from khoa and chhana such as burfi, peda, gulabjamun, milk cake, kalakand, rasogolla and sandesh. Each of these products has a unique flavour, texture and appearance (Pal & Raju, 2007). These products are important items in these societies and their religious functions. These products play a pivotal role in preserving buffalo milk and promoting its consumption among people, particularly in the high season of production. However, these products are prepared manually and often in unhygienic conditions without knowledge of the proper quality and conditions for specific products and without science-based knowledge and practices.

The most important factor in the promotion of traditional buffalo milk products is demographic change. For example, large immigrant populations have brought traditional ethnic flavours to mainstream food processing. Many people from buffalo milk-producing countries have settled in developed countries like the USA, the UK, Canada, Australia, Europe and the Middle East. So traditional

buffalo milk products are also becoming known in these countries. The global market potential and growth rate of traditional buffalo milk dairy products is strong. In view of increasing demand due to diminishing geographical and cross-cultural barriers, increasing globalisation trends, changing consumer preferences (particularly of middle class families in developing countries), increasing awareness of food safety and quality (particularly for children in developing countries), there is a strong need to develop new commercial pure buffalo milk-based products of the highest quality as exists for cow milk, with appropriate machinery and in hygienic and quality control environments. All these goals can be achieved, first, by efforts to make the buffalo milk sector more formalised and as organised as possible in high production areas; and second, by adopting optimum mechanisation, automation, international quality systems like Good Manufacturing Practice, Good Handling Practice, and Hazard Analysis and Critical Control Points by producers, processors, distributors, retailers and even consumers at every level of the dairy value chain.

In supermarkets and commercial stores of buffalo milk-producing countries, no particular buffalo milk products are currently available except mozzarella cheese (often being made on a large scale with cow milk or mixed milk). There are no specific processing lines and product range based on buffalo milk on an industrial scale and marketed internationally. In countries producing buffalo milk, mixed milk products, ranging from liquid milk to powdered milk, are available on the market. No specific buffalo milk ingredients are available as from cow milk. Buffalo and cow milk are mixed either before sale to the formal and informal sectors or is mixed by businesses. Most of the milk sold in milk shops in urban areas is actually mixed milk; even the most consumed hot dairy drink, 'milk tea', is made with mixed milk in restaurants and hotels, and mixed milk is served without knowing the proportions.

In many countries, buffalo milk is used to make traditional cheeses such as Mozzarella and Ricotta in Italy, Gemir in Iraq, Paneer and Cheddar in India and Pakistan, Domiati and salty cheeses in Egypt, Pecorino in Bulgaria and pickled cheeses in Middle Eastern countries. In Egypt, Laban rayeb (fermented milk) and zabadi (yoghurt) are also specialty products from buffalo milk. In Turkey, Afyon Kaymagi (clotted cream from pure buffalo milk produced in the Afyon province) is a specialty product made from buffalo milk.

According to Pandya and Khan (2006), when technologies already developed to make Western-type dairy cow milk products are applied to buffalo milk, the latter is not regarded as a suitable raw material owing to basic differences in composition and physicochemical properties. A thorough understanding of the properties of this milk and some modifications in the developed technologies are necessary to produce products of the same quality as exist for cow milk.

24.2 MAJOR MILK CONSTITUENTS AND THEIR NUTRITIONAL IMPORTANCE

Milk is considered a nutritionally complete food. It contains all the macronutrients and micronutrients necessary to sustain life in the neonate and the young infant, and adds quality to the human diet. The nutritional value of milk is also an important energy source. Consumption patterns for milk and evidence for its nutritional value, especially in children, vary from country to country, depending on their total production and priorities. Buffalo milk consumption can be beneficial for conditions such as atherosclerosis, milk allergy, anaemia, and dental problems, among others. Preparation of new types of buffalo milk as an alternative, designed to offset certain difficulties encountered with cow milk, have now been evaluated in humans (Lee & Lorenz, 1978). A large number of studies have focused on cow milk, even if the milk produced by other animals such as buffalo is essential in the human diet in different parts of the world. Buffalo milk and colostrum, like those of cow milk, also contain major and minor health beneficial components (Howarth *et al*., 1996). Typical average buffalo milk characteristics and composition are shown in Table 24.3. All the major components, like fat, protein, lactose and minerals, are higher in concentration in buffalo milk than in cow, goat, human and camel milk. Cumulatively, the higher total solids content than that of other mammalian milks make buffalo milk more nutritious.

24.2.1 Fat

Buffalo milk has about double the concentration of fat compared with cow milk (Pandya & Khan, 2006) and is mainly responsible for its high energy and nutritive value. Asker *et al*. (1974) found the fat content in buffalo milk to be between 6.9 and 8.5%, with an average of 7.4% (Menard *et al*., 2010) and 8.3% (Varrichio *et al*., 2007) but it can also reach 15% under normal conditions. Tonhati *et al*. (2011) analysed buffalo milk fat composition to verify the activity of Δ^9-desaturase in the mammary gland and to estimate additive genetic variances for fat as a function of herd, year, calving season, age at calving, lactation length and environmental effects. According to these authors, the mean fat yield was 90.1 ± 24.6 g/kg. Heritability estimates for fat were 0.26 ± 0.11 with enough additive genetic variation for fat yield to improve these traits through selection. Medhammar *et al*. (2011) also found interbreed differences in total fat in buffalo, yak, mare and dromedary camel milks as well as in mineral content.

Table 24.3. Characteristics and constituents of buffalo and cow milk.

Characteristics	Buffalo milk	Cow milk
pH	6.81[††]	6.76[††]
Acidity (D°) (1°D = 0.1 g/L)	16.2[††]	15.3[††]
Urea (mg/L)	237[††]	304[††]
Freezing point (°C)	–0.526[††]	–0.521[††]
Heat coagulation time	8 min 48 s[††]	6 min 30 s[††]
Total cholesterol (mg/100 g)	275[§]	330[§]
Free cholesterol (mg/100 g)	212[§]	280[§]
α-Tocopherol (μg/kg)	334[§]	312[§]
Energy (kcal/kg)	1035[‡]	701[‡]
Colour		
White (L) (a.u.)	74[††]	73[††]
Green (–a) (a.u.)	–1.6[††]	–2.0[††]
Yellow (b) (a.u.)	5.6[††]	7.4[††]
Constituents		
Water (g/kg)	832[†]	872[†]
Total solids (g/kg)	174.5[††]	136.7[††]
Fat (g/kg)	70.0[††]	41.0[††]
Solids-not-fat (g/kg)	104.5[††]	95.7[††]
Lactose (g/kg)	52.1[††]	48.0[††]
Protein (g/kg)	43.5[††]	33.5[††]
Non-casein nitrogen (g/kg)	8.9[††]	7.4[††]
Non-protein nitrogen (g/kg)	1.7[††]	1.6[††]
Casein (g/kg)	34.6[††]	26.1[††]
Whey protein (g/kg)	6.2[††]	5.8[††]
Ash (g/kg)	8.4[††]	7.7[††]
Fatty acids (% weight of total methyl esters; mean values)		
C4:0	2.80[‡‡]	2.53[‡‡]
C6:0	1.85[‡‡]	2.10[‡‡]
C8:0	1.08[‡‡]	1.35[‡‡]
C10:0	1.80[‡‡]	2.52[‡‡]
C12:0	2.3[‡‡]	2.87[‡‡]
C14:0	11.77[‡‡]	11.14[‡‡]
C14:1 *cis*-9	0.73[‡‡]	1.05[‡‡]
C15:0	1.74[‡‡]	1.16[‡‡]
C15:1 *cis*-10	0.36[‡‡]	0.27[‡‡]
C16:0	36.02[‡‡]	33.80[‡‡]
C16:1 *cis*-9	1.91[‡‡]	1.59[‡‡]
C17:0	0.84[‡‡]	0.61[‡‡]
C17:1 *cis*-10	0.30[‡‡]	0.24[‡‡]
C18:0	9.85[‡‡]	11.07[‡‡]
C18:1 *trans*-6 + *trans*-7 + *trans*-8 + *trans*-9	0.4[‡‡]	0.31[‡‡]
C18:1 *trans*-10	0.17[‡‡]	0.18[‡‡]
C18:1 *trans*-11	2.00[‡‡]	1.43[‡‡]
C18:1 *trans*-12	0.14[‡‡]	0.17[‡‡]
C18:1 *cis*-9	20.25[‡‡]	22.12[‡‡]
C18:2 *cis*-9, *cis*-12 (ω6)	0.93[‡‡]	1.34[‡‡]
C18:3 *cis*-9, *cis*-12, *cis*-15 (ω3)	0.72[‡‡]	0.61[‡‡]
C20:0	0.22[‡‡]	0.17[‡‡]
C18:2 *cis*-9, *trans*-11 (main CLA)	0.90[‡‡]	0.70[‡‡]

(*Continued*)

Table 24.3. (*Continued*)

Characteristics	Buffalo milk	Cow milk
Saturated fatty acids	70.8‡‡	69.6‡‡
Unsaturated fatty acids	29.2‡‡	30.4‡‡
ω6/ω3	1.3‡‡	2.2‡‡
C18:1 *trans*	2.70‡‡	2.09‡‡
Total *trans* (C18:1 *trans* + CLA)	3.61‡‡	2.79‡‡
Characteristics of fat globules		
Size (μm)	5††	4††
Charge (mV)	–14††	–11††
Polypeptides		
α_{s1}-Casein (g/kg)	14.4–18†	12–15†
α_{s2}-Casein (g/kg)	2.2–2.8†	3.0–4.0†
β-Casein (g/kg)	12.6–15.8†	9.0–11.0†
κ-Casein (g/kg)	4.3–5.4†	3.0–4.0†
γ-Casein (g/kg)	1.6†	1.0–2.0†
β-Lactoglobulin (g/kg)	3.9†	2.0–4.0†
α-Lactalbumin (g/kg)	1.4†	1.0–1.5†
Proteose peptone (g/kg)	3.3†	6.0–18†
Serum albumin (g/kg)	0.3†	1.0–4.0†
Lactoferrin (g/kg)	0.3†	0.05†
Non-protein nitrogenous compounds		
Amino acid (mg N/100 g)	5.13†	3.74†
Creatinine (mg N/100 g)	0.370†	0.49†
Creatine (mg N/100 g)	0.915†	3.93†
Uric acid (mg N/100 g)	0.244†	2.28†
Ammonia (mg N/100 g)	0.260†	0.67†
Undetermined (mg N/100 g)	9.30†	8.81†
Total amino acids		
Lysine (g/kg)	3.51¶	3.14¶
Histidine (g/kg)	1.66¶	1.49¶
Arginine (g/kg)	1.17¶	1.43¶
Aspartic acid (g/kg)	2.94¶	2.64¶
Threonine (g/kg)	1.22¶	1.05¶
Serine (g/kg)	0.72¶	0.58¶
Glutamic acid (g/kg)	9.96¶	8.51¶
Proline (g/kg)	4.44¶	3.42¶
Glycine (g/kg)	0.81¶	0.67¶
Alanine (g/kg)	1.57¶	1.34¶
Valine (g/kg)	2.52¶	2.41¶
Methionine (g/kg)	0.62¶	0.61¶
Isoleucine (g/kg)	2.48¶	1.98¶
Leucine (g/kg)	4.24¶	3.68¶
Tyrosine (g/kg)	0.48¶	0.53¶
Phenylalanine (g/kg)	2.31¶	1.89¶
Characteristics of casein micelles		
Size (nm)	190††	180††
Charge (mV)	–20††	–20††
Hydration (g H_2O/g dry pellet)	1.9††	2.2††

Table 24.3. (*Continued*)

Characteristics	Buffalo milk	Cow milk
Major minerals and trace elements		
Calcium (total/diffusible/colloidal) (mmol/L)	47.1/8.2/38.9[††]	30.5/8.6/21.9[††]
Magnesium (total/diffusible/colloidal) (mmol/L)	7.3/3.5/3.8[††]	4.6/3.0/1.6[††]
Sodium (total) (mmol/L)	20.3[††]	17.5[††]
Potassium (total) (mmol/L)	28.7[††]	42.0[††]
Chloride (total) (mmol/L)	16.6[††]	21.8[††]
Phosphate (total/diffusible/colloidal) (mmol/L)	27.7/9. 2/18.5[††]	19.2/9.9/9.3[††]
Citrate (total/diffusible/colloidal) (mmol/L)	8.3/7.1/1.2[††]	8.8/8.2/0.6[††]
Boron (μg/dL)	52–145[†]	27[†]
Cobalt (μg/dL)	0.69–1.61[†]	0.6[†]
Copper (μg/dL)	7–21[†]	13[†]
Iron (μg/dL)	42–152[†]	45[†]
Manganese (μg/dL)	38.2–65.8[†]	22[†]
Sulphur (μg/dL)	15 700–31 400[†]	30 000[†]
Zinc (μg/dL)	147–728[†]	390[†]
Enzymes		
Lipase (U/mL)	0.16–1.13[†]	0.1–0.6[†]
Alkaline phosphatase (U/mL)	0.12–0.18[†]	0.08–0.12[†]
Xanthine oxidase (U/mL)	0.075[†]	0.092[†]
Lysozyme (μg/mL)	0.152[†]	0.18[†]
Lactoperoxidase (U/mL)	5.2–9.8[†]	4.36–7.16[†]
Ribonuclease (μg/mL)	9.78[†]	8.23[†]
Protease (U/mL)	0.78[†]	0.68[†]
Vitamins		
Vitamin A (IU/mL)	340[†]	136[†]
Thiamine (mg/L)	0.2–0.5[†]	0.2–0.8[†]
Riboflavin (mg/L)	1.59[†]	1.7[†]
Pyridoxine (mg/L)	3.25[†]	0.67[*]
Ascorbic acid (mg/L)	0.67[†]	0.17–0.28[†]
Tocopherol (μg/g)	334.2[†]	2.99[**]

Sources: *Hodson (1944); [†]Sahai (1996); [‡]El-Agamy *et al*. (1998); [§]Zicarelli (2004a); [¶]Aliyev *et al*. (2005); **Kalioglu *et al*. (2009); [††]Ahmad (2010); [‡‡]Menard *et al*. (2010).

24.2.1.1 Fat globules

Milk fat is secreted in the form of globules initially surrounded by a membrane, the milk fat globule membrane, which maintains the integrity of the globules and renders them compatible with the aqueous environment (Keenan & Patton, 1995). Buffalo milk fat globules consist almost entirely of triglycerides, whereas membranes contain mostly complex lipids of greater size than cow milk. The larger size of buffalo fat globules (5 vs. 3.5 μm) is related to the higher amount of fat in buffalo milk (Ahmad *et al*., 2008a; Menard *et al*., 2010). The size distribution of fat globules varies between dairy species (Mehaia, 1995; El-Zeini, 2006; Pandya & Khan, 2006). Buffalo and cow milk fat globule membranes contain the same classes of polar lipids, phosphatidylethanolamine, sphingomyelin and phosphatidylcholine being the main constituents. This is of major interest; some studies have reported a larger number of small fat globules in buffalo milk than in cow and sheep milk (Zicarelli, 2004a). It is well known that small fat globules are rich in polyunsaturated fatty acids. A significantly higher percentage of phosphatidylcholine and lower percentage of sphingomyelin are found in buffalo milk (Menard *et al*., 2010). The fat globule charge is also found to be higher in buffalo milk than in cow milk (Table 24.3). Evidently, the greater size fat globules have more charge around the greater surface area.

24.2.1.2 Triglycerides

Buffalo milk fat has been classified into high, medium and low molecular weight triglycerides on the basis of average molecular weight of the constituent fatty acids, with average values of 42.5%, 17.1% and 40.5%, respectively (Arumughan & Narayanan, 1982). The triglyceride fractions of buffalo milk are further resolved into saturated, *trans*-monoene, *cis*-monoene, diene and polyene triglyceride species. Although buffalo milk fat contains more lipids, the proportions of monoglycerides, diglycerides and triglycerides are similar in buffalo and cow milk; although quantitative variations exist in both, qualitatively the fat of both milks seems to be similar (Ramamurthy & Narayanan, 1974a, b). The pattern of fatty acids forming various triglyceride classes of milk fat also appears to be similar for both species. Buffalo milk fat has a higher melting point than that of cow milk due to the higher proportion of saturated fatty acids (Achaya & Banerjee, 1946; Ramamurthy & Narayanan, 1971; Menard *et al*. 2010). Lakshminarayana (1983) found that temperatures around 23°C are critical for the majority of triglycerides to crystallise, and maximum yield is obtained at the crystallisation temperature of 31°C, which is approximately the melting point of buffalo milk fat. The amount of liquid fraction is inversely proportional to the initial melting points of milk fat of both buffalo and cow. The size of the triglyceride crystals is higher in fractions obtained at higher temperatures than at lower temperatures. Solid fractions show markedly different melting points for the different fatty acid compositions. The short-chain triglycerides contribute to low melting point triglycerides while long-chain triglycerides contribute to high melting point triglycerides. As the temperature of fat clarification increases from 100 to 120°C, the size and quantity of fat grains increases. The size and quantity of grains is much higher in buffalo milk fat than in cow milk fat. The optimum incubation temperature for large grains is 30°C. The emulsifying power of buffalo milk fat is better because of a higher percentage (50%) of butyric acid (C4) in triglycerides compared with only 37% in cow milk. This is the reason for higher butter and ghee yields from buffalo milk. The higher percentage (9–12%) of high melting point triglycerides provides larger fat grains, which in turn impart a grainy feel to ghee made from buffalo milk. Aside from this, buffalo milk ghee is less prone to hydrological rancidity than cow milk ghee (Malik, 2011).

24.2.1.3 Fatty acids

Fatty acid analyses revealed that saturated fatty acids, mainly butyric, myristic, palmitic, *trans* fatty acids, linolenic (ω3) and conjugated linoleic acid are higher in buffalo milk than in cow milk (Table 24.3). Some authors have reported changes in fatty acid composition according to breed (Talpur *et al*., 2007), stage of lactation (Arumughan & Narayanan, 1981), season (Asker *et al*., 1978; Talpur *et al*., 2008) and diet (Patiño *et al*., 2008) (Table 24.3). Differences are present not only between species but also within species. Talpur *et al*. (2007) studied two major buffalo breeds in Pakistan, namely Nili-Ravi and Kundi, and found milk fat from Kundi buffaloes to contain significantly lower amounts of saturated fatty acids than that from Nili-Ravi buffaloes (66.96 vs. 69.09 g/100 g, respectively), while mean monounsaturated fatty acid (MUFA) content (27.62 vs. 25.20 g/100 g) and total *trans* fatty acids (3.48 vs. 2.48 g/100g of fatty acids) were significantly higher in the milk fat of Kundi buffaloes. Fatty acids undergo different metabolic fates depending on their chain length and degree of saturation. The metabolic discrimination between varying fatty acids begins in the gastrointestinal tract, with medium-chain fatty acids being absorbed more efficiently than long-chain fatty acids. These differences in metabolic handling mean that medium-chain fatty acids have the potential to be weight loss agents (Papamandjaris *et al*., 1998) for which buffalo milk is a potential source.

24.2.1.4 Conjugated linoleic acid

The average content of conjugated linoleic acid (CLA) in buffalo milk ranges from 4.4 to 7.6 mg/g fat. Buffalo milk contains a significantly higher amount of rumenic acid (C18:2 *cis*-9, *trans*-11, the main CLA) than cow milk. This is not surprising since C18:1 *trans*-11 is the precursor of C18:2 *cis*-9, *trans*-11, formed in the mammary gland by Δ^9-desaturase (Menard *et al*., 2010). Tonhati *et al*. (2011) analysed buffalo milk fat composition for *cis*-9, *trans*-11 CLA; the average was 0.69 ± 0.16% and the heritability estimate for CLA percentage was 0.35 ± 0.14, showing that *cis*-9, *trans*-11 CLA percentage can be enhanced by selection in buffaloes and can greatly contribute to improving human health. The average human intake of CLA varies among countries, and different CLA isomers exhibit different biological effects. CLA has been shown to inhibit the growth of some cancers, including prostate, stomach and particularly breast cancer, in humans. Buffalo milk contains higher levels of CLA than cow milk so it can be nutritionally and medicinally valuable against such diseases.

24.2.1.5 Minor fat constituents (cholesterol, phospholipids, gangliosides)

The cholesterol content of buffalo milk is about 0.65 g/L whereas for cow milk it is 3.14 g/L (Rajorhia, 1988; Ganguli, 1992; Zicarelli, 2004a). Buffalo milk contains significantly lower amounts of polar lipids per gram of lipids (0.26% vs. 0.36%) than cow milk, but significantly higher amounts of polar lipids per litre of milk (+26%).

Phospholipids are also lower in buffalo milk. Buffalo milk is unique in having gangliosides (Berger *et al*., 2005) that are not contained in bovine milk, such as gangliosides that belong to the GM1 class. Furthermore, buffalo milk has unknown gangliosides F and L. Gangliosides are a different class of sphingolipids that contain one or several sialic acid moieties. Immunoactive gangliosides can be present in high amounts in buffalo milk. Milk serum from buffaloes, for example derived from Mozzarella cheese production, contains specific gangliosides in the same amounts as human milk, which makes it suitable for humanisation of infant formulae. It has been found that buffalo milk and human milk have comparable GM1 and polysialoganglioside levels. Sphingomyelin has been demonstrated to reduce the risk of colon cancer in animal studies. Colarow *et al*. (2003) evaluated gangliosides in Swiss cow milk (SCM), Italian buffalo milk (IBM) and its serum, Pakistan buffalo colostrum (PBC), Pakistan buffalo mature milk (PBM) and Pakistan buffalo milk from rice-growing areas (PBR). The dairy gangliosides were determined as lipid-bound sialic acid (LBSA). Molar ratios of LBSA in the hydrophilic and lipophilic ganglioside fractions were 52 : 48 to 79 : 21, respectively. Mature buffalo milk types had 40–100% more LBSA in the lipophilic ganglioside fraction than SCM. Liquid PBC was higher in LBSA (24 nmol/g) than mature milk types (8–11 nmol/g). Lipophilic gangliosides (but importantly not hydrophilic gangliosides) from IBM and its serum decreased prostaglandin series 2 production by 75–80% in cultured human colonic epithelial cells exposed to tumour necrosis factor (TNF)-α. Hydrophilic GD(3) and lipophilic GM(3) selectively bound rotavirus particles prepared from a rhesus strain and its mutant. One ganglioside fraction in IBM showed a GM1-specific binding to cholera toxin subunit B (CTB). IBM serum (IBMS) was a rich source of LBSA (420 nmol/g protein). Human and Italian buffalo milk had similar CTB binding, and both had increased polysialogangliosides compared with cow milk. The toxin-binding properties of buffalo milk gangliosides and the anti-inflammatory activity of the lipophilised ganglioside fraction could be important for developing innovative food applications.

24.2.2 Proteins

Buffalo milk, like cow milk, contains two major protein groups distinguished by their solubility in unheated milk at pH 4.6 and 20°C: caseins (insoluble) and whey proteins (soluble). Both groups have unique physiochemical and biological properties. Of the proteins in buffalo milk, about 80% are caseins and about 20% are whey proteins and non-protein nitrogen, similar to cow milk (Laxminarayana & Dastur, 1968a, b; Sirry *et al*., 1984; Sahai, 1996; Ahmad *et al*., 2008a; Ahmad, 2010). Sindhu and Roy (1973) reported 41.3 ± 0.4 g/kg and Ahmad *et al*. (2008a) 43.5 ± 3.4 g/kg of total proteins in buffalo milk, respectively, while Tonhati *et al*. (2011) reported 56.9 ± 15.2 g/kg with heritability estimates for protein of 0.25 ± 0.11. Total amino acid composition of buffalo and cow milk showed that the quantity of almost all amino acids was higher in buffalo milk, except arginine and tyrosine, than in cow milk (Table 24.3). Glutamic acid, proline, leucine and lysine are the most abundant amino acids in both milks, but quantitative differences exist between both milks. The quantitative differences may be related to the high protein concentration in buffalo milk, but percentages of almost all amino acids are similar in both milks showing that they are nutritionally equal (Aliyev *et al*., 2005; Ahmad, 2010). Hinz *et al*. (2012) performed proteomic analysis of bovine, caprine, buffalo, equine and camel milk and highlighted significant interspecies differences. Comparisons showed that buffalo milk contained higher amounts of amino acids, except for arginine, and that amino acid sequences exhibited 95% homology (Fig. 24.2). Table 24.4 illustrates the accession number of individual protein classes of buffalo milk with their identification methods, milk fractions, localisation and functions.

24.2.2.1 Caseins

Caseins exist primarily as calcium phosphate stabilised micellular complexes in buffalo milk, as in other mammalian milk. Caseins are a heterogeneous family of proteins predominated by α_{s1}–, α_{s2}–, β– and κ-casein (Eigel *et al*., 1984; Feligini *et al*., 2009; Ahmad, 2010). Individual casein proteins are small molecules with molecular mass of 20–25 kDa, and primary amino acid sequences that are high in proline content. Proline prevents casein molecules from having much secondary structure (α-helices, β-sheets and β-turns), which is higher in buffalo milk than in cow milk. Caseins are relatively hydrophobic, and have primary sequence clusters with high surface hydrophobicity that contributes to functional properties such as emulsification and foaming. Fox (2001) and Wong *et al*. (1996) reviewed the structure and function relationship of individual caseins. The caseins in buffalo milk are subclassified into α_{s1}, α_{s2}, β and κ. Buffalo whole caseins contain α_s 45.6%, β 43.4% and κ 11.0% (Nagasawa *et al*., 1973; Högberg & Lind, 2003; Zicarelli, 2004b; Ahmad, 2010). Feligini *et al*. (2009) found the mean values of casein fraction concentrations in individual buffalo milk samples to be 8.9 g/L with a relative standard deviation (RSD) of 20% for α_{s1}-casein, 5.1 g/L with RSD of 25% for α_{s2}-casein, 20.9 g/L with RSD of 16% for β-casein, and 4.1 g/L with RSD of 24% for κ-casein. These caseins, like cow caseins, form supramolecular colloidal structures called casein micelles (Ganguli, 1973; Ahmad, 2010). Buffalo milk contains a negligible proportion of soluble casein (0.03 g/dL), about 1% of the total

		4	14	42	74	115	119	148	174	192				
α_{s1}-Casein	Buffalo	Gln	Gly	Thr	Ile	Leu	Gln	Gln	Pro	Gly				
	Cow	His	Glu	Lys	Asn	Ser	Arg	Glu	Thr	Gln				
		2	29	44	147	157	170	175	176	182	199			
α_{s2}-Casein	Buffalo	His	His	Ile	Ile	Asp	His	Thr	Try	Try	Asn			
	Cow	Asn	Asn	Val	Phe	Glu	Arg	Ala	Leu	His	Lys			
		25	41	57	84	108	164							
β-Casein	Buffalo	His	Met	Met	Lys	Ile	Pro							
	Cow	Arg	Thr	Thr	Asn	Val	His							
		14	19	80	96	126	128	138	140	147	149	156	162	168
κ-Casein	Buffalo	Glu	Asn	Pro	Thr	Val	Val	Ile	Asn	Ala	Ser	Val	Ala	Val
	Cow	Asp	Ser	Ser	Ala	Ala	Gly	Val	Ser	Asp	Pro	Pro	Val	Ala
		1	163											
β-Lactoglobulin	Buffalo	Ile	Val											
	Cow	Leu	Ile											
		17												
α-Lactalbumin	Buffalo	Asp												
	Cow	Gly												

Figure 24.2. Differences in amino acid sequence of major proteins of buffalo and cow milk.

Table 24.4. Buffalo (*Bubalus bubalis*) milk proteins with their accession number on Swiss-Prot with secreted protein localisations.

Protein	Accession number	Identification method*	Milk fraction†	Function‡
α_{s1}-Casein	O62823	PMF, MS-MS	S, W, MFGM	GT
α_{s2}-Casein	Q3Y443	PMF, MS-MS	S, W	GT
β-Casein	Q9TSI0	PMF	S, W	GT
κ-Casein	P11840	PMF, MS-MS	S, W, MFGM	GT
α-Lactalbumin	Q9TSN6	PMF, MS-MS	S, W	E
β-Lactoglobulin	P02755	PMF, MS-MS	S, W, MFGM	GT
Lactoperoxidase	A5JUY9	MS-MS	W	E, DI
Lactotransferrin	O77698	MS-MS	W	DI
Mammary-derived growth inhibitor, fatty acid-binding protein	Q5XLB1	PMF, MS-MS	S, W, MFGM	FTM

* MS-MS, tandem mass spectroscopy; PMF, peptide mass fingerprinting.

† S, skimmed milk; W, whey; MFGM, milk fat globule membrane.

‡ DI, defence/immunity; E, enzyme; FTM; fat transport/metabolism; GT, general transport.

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casein, unlike cow milk with 0.11 g/dL and about 5% of the total casein. The ratio of micellar to soluble casein in buffalo milk was found to be 91 versus 21 for cow milk (Ganguli, 1973). A variety of molecular forces are involved in this structure, and the stability of the casein micelle depends on a balance between electrostatic repulsion and hydrophobic interaction. Moreover, micellar calcium phosphate cross-links the casein molecules and neutralises negatively charged phosphoseryl groups, allowing the formation of hydrophobic interactions between caseins. Micellar size, hydration, zeta potential and mineralisation are the most described physicochemical characteristics of casein micelles. The casein micelles of buffalo milk are bigger (Ganguli, 1973; Sood *et al*., 1976; Sirry *et al*., 1984; Ahmad, 2010), less hydrated (Kuchroo & Malik, 1976; Ahmad *et al*., 2008a, b; Ahmad, 2010) and with more colloidal calcium and inorganic phosphate (Sabarwal *et al*., 1972; Rajput *et al*., 1983; Sahai, 1996; Ahmad *et al*., 2008a, b; Ahmad, 2010) than cow milk casein micelles. Varindra *et al*. (1994) found the voluminosity and solvation of casein

micelles in skimmed buffalo milk to be 3.88 mL/g and 3.18 g H_2O/g of dry pellet at room temperature (Table 24.3). All caseins show genetic polymorphism and have post-translational modification with either phosphate (phosphorylation) and/or carbohydrate (glycosylation) moieties.

24.2.2.1.1 α_s-Caseins

Whole casein of buffalo milk contains two α_{s1}- and two α_{s2}-casein fractions. The two α_{s1}-casein fractions seem to have identical peptide chains as do the two α_{s2}-casein fractions, with the individual fraction within each group differing only in their phosphate content. α_{s1}- and α_{s2}-caseins are differently phosphorylated. α_{s1}-Casein shows no polymorphism whereas the electrophoretic pattern of α_{s2}-casein suggests genetic polymorphism (Addeo *et al*., 1977). Abd El-Salam (1975) found heterogeneous α_s-casein in buffalo milk giving four electrophoretic components along with their different electrophoretic mobilities: α_{s1} (1.08), α_{s2} (1.06), α_{s3} (1.03) and α_{s4} (0.99). He also found that the α_s fraction contains only two major components of buffalo α_s-casein, namely α_{s2} (1.06) and α_{s3} (1.03), which comprise 90% of the total α_s-casein whereas the other two, namely α_{s1} (1.08) and α_{s4} (0.99), which are the fastest and slowest components, are present in small quantities. This heterogeneity of α_s-casein in buffalo milk has been reported by Aschaffenburg *et al*. (1968), who found five components in this fraction, and Nagasawa *et al*. (1973), who found that the buffalo α_s fraction comprised three or four components with lower mobility than cow α_s-casein. Feligini *et al*. (2009) also found heteromorphic α_{s1}-casein in several individual buffalo milk samples as there were two peaks with molecular masses of 23.49 and 23.52 kDa. Feligini *et al*. (2009) detected only one form for α_{s1}-casein with molecular mass of 22.74 kDa. The amino acid composition of cow α_{s1} B variant is similar to that of the α_s fraction of buffalo milk but not identical (Fig. 24.2). The most pronounced differences are the high content of proline and threonine in α_s-casein of buffalo milk and the low content of the basic amino acids. Nagasawa *et al*. (1973) also found a low content of the basic amino acids in buffalo α_s-casein. The α_s-casein of buffalo milk had higher phosphorus content, namely 1.2% versus 1.05% quoted for the cow α_{s1} B variant. α_s-Casein of buffalo milk is almost free of carbohydrate. In addition to this, cysteine and cystine are also absent from α_s-casein of buffalo milk. Solubility of α_s-casein of buffalo milk is a function of increased $CaCl_2$ concentration and temperature, and is more soluble at lower temperatures. α_s-Casein of buffalo milk is less soluble to Ca^{2+} than cow α_s-caseins (Abd El-Salam, 1975). According to the amino acid sequences of sheep, goat and buffalo α_{s1}-casein, peptides corresponding to residues 99–109 have 10 of the 11 amino acids that are identical to residues 99–109 of bovine α_{s1}-casein. Because this peptide was derived from digestion of bovine casein with pepsin, it might be released in the stomach and contribute to protection against microbial infection in the gastrointestinal tract (López-Expósito & Recio, 2008).

24.2.2.1.2 β-Caseins

β-Casein from buffalo milk behaves as a single homogeneous protein with a mobility identical to the B variant of cow β-casein as reported by Abd El-Salam and El-Shibiny (1975). Feligini *et al*. (2009) also found only one form for β-casein with molecular mass of 24.03 kDa. Buffalo β-casein possesses identical end-groups to those of cow β-casein, namely N-terminal arginine and assuming a single polypeptide chain a possible C-terminal sequence of Ile-Ile-Val-OH (Kalan *et al*., 1965). However, the amino acid composition and the tryptic peptide pattern of the two proteins are not the same (Abd El-Salam & El-Shibiny, 1975). The amino acid pattern of buffalo β-casein is characterised by lower histidine, arginine, valine and leucine but higher proline and methionine content (Fig. 24.2). Arginine was the only amino-terminal amino acid in buffalo β-casein (Abd El-Salam & El-Shibiny, 1975). The amino acid sequence of the first 22 residues and three C-terminal ones are identical in both species. Buffalo β-casein contains only four phosphate groups instead of the five found in the cow protein. β-Casein showed no polymorphism (Addeo *et al*., 1977). One of the most important findings is the presence of bioactive peptides in the enzymatic hydrolysates of β-casein: β-casein morphopeptides, mineral-binding peptides, antihypertensive peptides, immune-stimulating peptides and surface-active peptides (Abd El-Salam, 1992). Owing to the presence of the milk endopeptide enzyme plasmin, β-casein can be degraded into N- and C-terminal peptides with different properties.

24.2.2.1.3 κ-Caseins

Feligini *et al*. (2009) found κ-caseins as heteromorphic in several individual milk samples, showing three peaks with molecular masses of 19.17, 19.18 and 19.25 kDa. Addeo *et al*. (1977) fractionated buffalo milk κ-casein into seven components (κ1–κ7). They obtained 12 pure components: κ1, κ2, κ3, κ4a, κ4b, κ5a, κ5b, κ6, κ7a, κ7b, κ7c and κ7d. Fraction κ1, the main buffalo κ-casein fraction, which represented approximately 40% of the total κ-casein, is devoid of carbohydrates and it is similar to its cow counterpart. The κB_1-casein fraction in cow milk represents only 25% of the total κ-casein (Pujolle *et al*., 1966) and this explains why the total carbohydrate content of whole buffalo κB_1-casein is lower than that of its cow counterpart. κ-Casein showed no polymorphism (Addeo *et al*., 1977). The galactose, *N*-acetylgalactosamine and sialic acid contents of

buffalo κ-casein fractions ranged from 0 to 4.3, 5.5 and 8.5 mol/mol protein, respectively (Addeo *et al*., 1977). The maxima are higher than the corresponding figures in cow κ-casein fractions for sialic acid and galactosamine, and lower for galactose, with values ranging from 0 to 6.7, 3.5 and 4.3 mol/mol protein, respectively, for the seven fractions isolated from cow κA-casein. Identical subunits containing the three carbohydrates are attached at different places on the peptide chain of κ-casein (Pujolle *et al*., 1966). It was also revealed from the study of carbohydrate composition and amino acid sequence that carbohydrates are attached to a few residues of the caseinomacropeptide and from branched chains of different lengths.

24.2.2.2 Whey proteins

Whey proteins not only play an important role in nutrition as an exceptionally rich and balanced source of amino acids, but also exert specific physiological actions and impart many functional properties. Buffalo milk whey proteins are a heterogeneous polymorphic group of proteins composed of α-lactalbumin (ALA, 20%), β-lactoglobulin (BLG, 50%), serum albumin (10%), immunoglobulins (10%) and proteose peptones (<10%). Unlike caseins, whey proteins have high levels of secondary, tertiary and quaternary structure, and are typically heat-labile globular structures. All whey proteins contain intermolecular disulphide bonds that stabilise their structures. Whey proteins are not extensively glycosylated, and none is phosphorylated. The dominant proteins (ALA and BLG) are responsible for functional properties, predominantly foaming and gelation, that have been commercialised in whey protein concentrate and isolate products. Major whey proteins like BLG and ALA and the minor whey proteins lactoferrin, lactoperoxidase, lysozymes and immunoglobulins are nutritionally significant and are antimicrobial proteins whose concentrations can be seen in Table 24.3. Whey protein contains a high amount of essential amino acids, which makes it more nutritionally desirable than many other protein sources. Branched-chain amino acids are specific amino acids which are metabolised by muscle rather than liver. Branched-chain amino acids may aid muscle recovery and growth, and therefore are beneficial for athletes.

24.2.2.2.1 β-Lactoglobulin

The BLG level in buffalo milk is slightly higher than in cow milk. Buffalo BLG exists in solution as a dimer with an effective molecular mass of 36 kDa. Malik and Bhatia (1977) found that buffalo BLG exists as a dimer at pH 5.2 but converts into monomer at pH below 3.5 or above 6.5. At low temperature, near 0°C with pH 3.5, it exists as tetramer. BLG and its peptide fragments have a variety of useful nutritional and functional health benefits which make this protein of interest in the beverage (sports drink) industry. Buffalo BLG is homologous to the cow B variant (Fig. 24.2). No polymorphism was observed for BLG by Addeo *et al*. (1976), although Aschaffenburg *et al*. (1968) and Grosclaude *et al*. (1974) had shown the presence of two predominant genetic variants, A and B, of BLG in cow and buffalo milk, whereas Pellegrino *et al*. (1991) found that cow milk whey contains BLG A, which is absent in buffalo milk. Electrophoretic mobility of BLG is the same as for variant B of cow whey at either alkaline or acidic pH, which is probably an ancestral variant (Addeo *et al*., 1976). These globular proteins are more water soluble than caseins and are subject to heat denaturation. Native whey proteins have good gelling and whipping properties, but denaturation increases their water-holding/retention capacity. Denaturation of BLG occurs at pressures above 100 MPa, being nearly complete after treatment at 400 MPa and is completely associated with the casein micelles (Huppertz *et al*., 2005). BLG plays a role in the transport of retinol (vitamin A) around the body.

24.2.2.2.2 α-Lactalbumin

ALA is a major protein of the whey fraction of milk and possesses a number of biological features in addition to its role as a specifier protein in the lactose synthase system. The lactose synthase complex is responsible for the biosynthesis of lactose in the lactating mammary gland. This enzyme consists of two protein components: β-1,4-galactosyltransferase, the catalytic component, and ALA, the regulatory component. Even though functionally divergent, it is homologous to C-type lysozymes (Brew *et al*., 1967). It is a calcium-binding protein (Hiraoka *et al*., 1980) and binds different metal ions (Kronman & Fasman, 1989; Ren *et al*., 1993). The complete amino acid sequence of buffalo milk ALA differs only in one position from cow ALA at position 17 (Asp in buffalo ALA and Gly in cow ALA) (Fig. 24.2). The crystal structure of buffalo ALA showed another difference at position 27 (Ile) (Calderone *et al*., 1996). Addeo *et al*. (1976) reported that buffalo ALA differs from its cow B counterpart by a substitution Asn/Gly at position 17 and by another substitution, probably Glu/Gln or Asp/Asn, at an unknown position. ALA of buffalo milk shows no polymorphism. Denaturation of ALA occurs at pressures of 400 MPa, reaching greater than 90% after treatment at 800 MPa (Huppertz *et al*., 2005). ALA is used commercially to make infant formulae more similar to human milk. It binds calcium, zinc and other minerals. It may enhance immunity and reduce the risk of some cancers.

24.2.2.3 Minor proteins

Milk provides the neonate with not only nutrients but also a host of defence factors such as antibacterial, anti-inflammatory and immunomodulatory agents (Fig. 24.3).

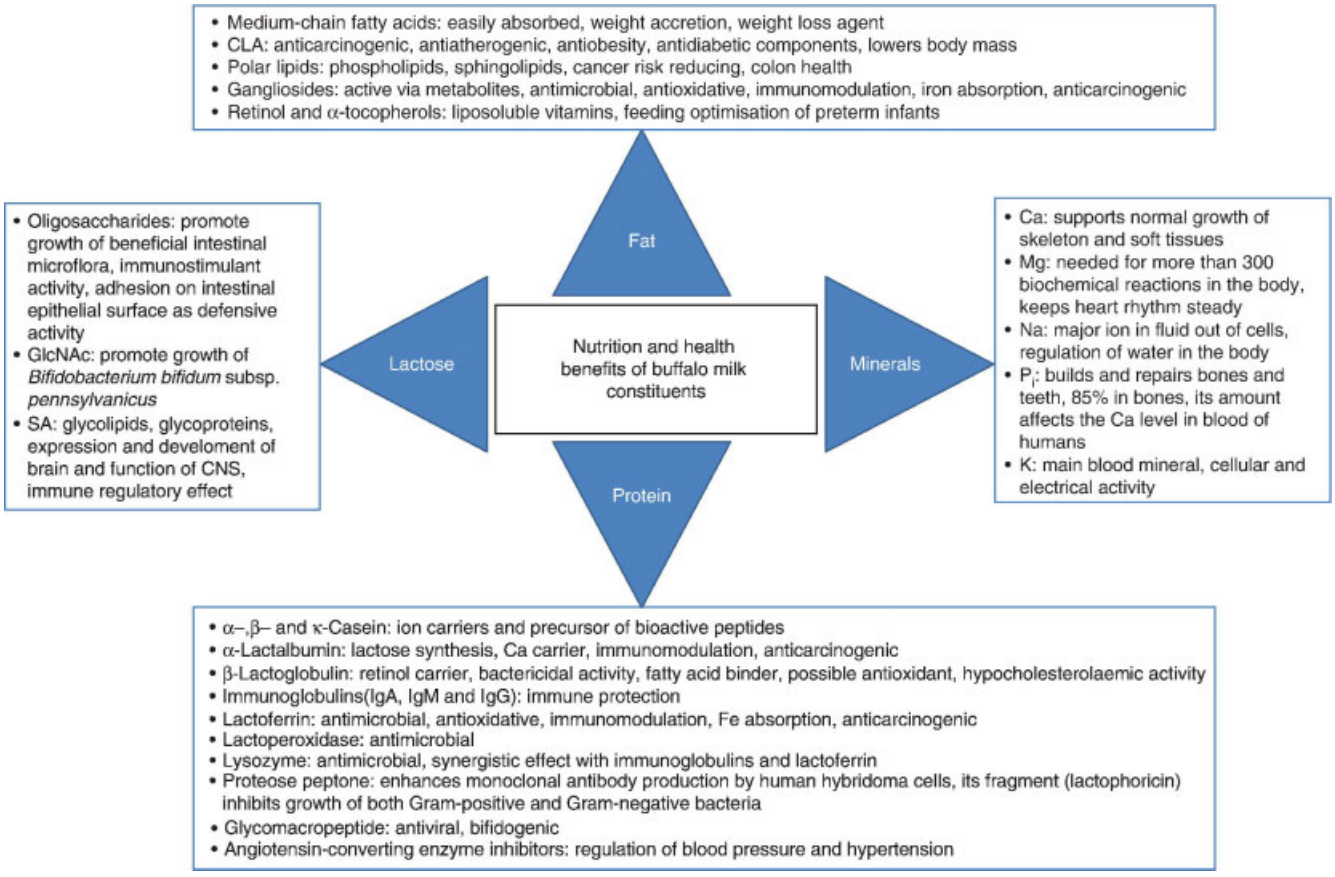


Figure 24.3. Summary of nutrition and health benefits of buffalo milk constituents. CLA, conjugated linoleic acid; GlcNAc, *N*-acetylglucosamine; SA, sialic acid.

Antimicrobial agents in milk include immunoglobulins, lactoferrin, antiviral lipids, vitamin-binding proteins and the enzymes lysozyme, lactoperoxidase and xanthine oxidase. Buffalo milk is regarded as a better health food than cow milk because of the presence of higher levels of various bioprotective factors, such as immunoglobulins, lactoferrin, lysozyme, lactoperoxidase and bifidogenic factors (Table 24.3), which render buffalo milk more suitable than cow milk for the preparation of a wide range of special dietary and health foods.

24.2.2.3.1 Proteose peptone

The level of proteose peptone (PP) in buffalo milk is higher (330.5 mg/L) than in cow milk (240.5 mg/L) (Sindhu & Singhal, 1988). Taha and Keilwein (1989) found that the nitrogen of PP in buffalo milk has a range of 19.0–35.0 g/100 g, with an average value of 26.9 g/100 g. This is about 3.6% of the total nitrogen in buffalo milk. Buffalo milk PP can be separated into three components, namely PP-3, PP-5 and PP-8, which have molecular masses of 25.12, 23.17 and 20.89 kDa, respectively. The three components contain the same 17 amino acids but in different concentrations. On further purification and polyacrylamide gel electrophoresis (PAGE), component PP-3 was resolved into four bands, while components PP-5 and PP-8 exhibited one band each (Ram & Joshi, 1990). PP in buffalo milk is highly glycosylated and these three components also showed significant differences in their phosphorus content (PP-3, 0.2%; PP-5, 1.5%; PP-8, 2.6%). In a very recent study by Pedersen *et al*. (2012), it was found that milk PP3 (also known as lactophorin) is a small phosphoglycoprotein that is exclusively expressed in the lactating mammary gland. A 23-residue synthetic peptide (lactophoricin, Lpcin S), corresponding to the C-terminal amphipathic α-helix of PP3, has previously been shown to permeabilise membranes and display antibacterial activity. Lactophorin readily undergoes proteolytic cleavage in milk and during dairy processing, and it has been suggested that PP3-derived peptides are part of milk's endogenous defence system against bacteria. In this study, the authors reported that a 26-residue C-terminal peptide (Lpcin P) can be generated by trypsin proteolysis of PP3 and that structural and functional studies of Lpcin P indicate that the peptide has antibacterial properties. Lpcin P showed an α-helical structure in both anionic and organic solvents, and the amount of α-helical structure was increased in the presence of lipid vesicles. Oriented circular dichroism showed that Lpcin P oriented parallel to the membrane surface. However, the peptide permeabilised calcein-containing vesicles efficiently. Lpcin P displayed antibacterial activity against *Streptococcus thermophilus*, but not against *Staphylococcus aureus* and *Escherichia coli*. The PP3 full-length protein did not display the same properties, which could indicate that PP3 functions as a precursor protein that upon proteolysis releases a bioactive antibacterial peptide. Further studies are required to exploit the PP fractions and their health benefits for buffalo milk.

24.2.2.3.2 Lactoferrin

Lactoferrin, a prominent iron-binding protein of the transferrin family found in milk, many other secretory fluids and white blood cells, is a monomeric glycoprotein. Crystallographic analyses of lactoferrin show differences among species. It has been suggested to have a number of functional properties including anticancer, antimicrobial, antiviral, antioxidant and immunomodulating effects on cell growth, and is also able to bind and inhibit bioactive compounds such as lipopolysaccharides and glycosaminoglycans and has toxin-binding properties (Baveye *et al*., 1999; Chierici, 2001). Its intense red colour is a most striking characteristic when incubated in the presence of Fe^{3+}, and was first fractionated as an unknown 'red fraction' from cow milk. These are single-chain polypeptides of about 80 kDa, containing one to four glycans depending on the species. Lactoferrin has been identified in buffalo milk at an average concentration of 0.32 mg/mL, higher than in cow milk, as shown in Table 24.3 (Abd El-Gawad *et al*., 1996; Sahai, 1996; Mahfouz *et al*., 1997). The *in vitro* activity of lactoferrin includes transcriptional activation of several genes. The purified active peptide from lactoferrin hydrolysate was named lactoferricin (Bellamy *et al*., 1992). In the natural state, bovine lactoferrin is only partly saturated with iron (15–20%) and has a salmon-pink colour, the intensity of which depends on the degree of iron saturation. Iron-depleted lactoferrin with less than 5% iron saturation is called apo-lactoferrin, whereas iron-saturated lactoferrin is referred to as holo-lactoferrin. The lactoferrin found in human milk is apo-lactoferrin, so the added lactoferrin in infant formulae must be of that nature.

The molecular mass of buffalo lactoferrin is 73.7–74 kDa and its metal binding sites and other properties are similar to those of cow lactoferrin. The four most abundant amino acids of buffalo milk lactoferrin are Lys, Glu, Asp and Leu, whereas in cow lactoferrin they are Glu, Asp, Leu and Ala. The carbohydrate moiety of buffalo lactoferrin contains 2.2–3.2 g of mannose, 1.7–1.9 g of *N*-acetylglucosamine, 0.4 g of sialic acid and 0.2 g of fucose per 100 g lactoferrin, and is similar to that of milk lactoferrin of Friesian and Brown-Swiss cattle (Mahfouz *et al*., 1997). The oral administration of lactoferrin has various benefits for animals and humans (Tomita *et al*., 2002; Wakabayashi *et al*., 2003; Wakabayashi, 2006). Orally administered lactoferrin enhances interleukin (IL)-18 production in intestinal epithelial cells and increases the numbers of CD4, CD8 and

natural killer cells in intestinal mucosa (Kuhara *et al*., 2000; Wang *et al*., 2000). Over 60% of administered bovine lactoferrin survives passage through the adult human stomach and enters the small intestine intact (Kuwata *et al*., 1998, 2001; Troost *et al*., 2001). The cationic N-terminus of bovine lactoferrin is of special interest because of its reported antibacterial activity (Bellamy *et al*., 1992). Lactoferrin in external secretions like tears, saliva and seminal fluids, as well as in milk, suggests that it has a role in defence against invading pathogens. Its broad antimicrobial spectrum includes Gram-positive and Gram-negative bacteria, yeasts and fungi, with added antiviral activity against cytomegalovirus, herpes, influenza, HIV, rotavirus and hepatitis C (Kawasaki *et al*., 1993; Teraguchi *et al*., 1994, 1995; Superti *et al*., 1997). Ingestion of bovine lactoferrin for 8 weeks decreased serum hepatitis C virus RNA levels in chronic hepatitis C patients with low viral loads (Tanaka *et al*., 1999). Treatment with bovine lactoferrin for 7 days in adults aided in the eradication of *Helicobacter pylori* gastric infection (Di Mario *et al*., 2003). Several animal studies have reported that lactoferrin can inhibit the development and progression of colonic tumours in rats (Sekine *et al*., 1997a, b), besides having chemopreventive effects on cancers in the oesophagus, lung, tongue, bladder and liver.

Anti-mould activity of buffalo and cow lactoferrin has been studied against four test strains (*Aspergillus niger*, *Rhizopus oryzae*, *Penicillium roquefortii* and *Penicillium camemberti*). The lowest effective inhibitory concentration of buffalo and cow lactoferrin was in the range 25–75 and 25–125 µg/mL, respectively (Shilpa *et al*., 2005). The antifungal activity of buffalo lactoferrin against five yeast strains (*Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, *Rhodotorula glutinis*, *Aspergillus niger* and *Candida guillermondi*) has been studied. The lowest effective inhibitory concentration of buffalo lactoferrin was in the range 25–250 µg/mL. Among all yeast cultures *R. glutinis* and *A. niger* were most sensitive to buffalo lactoferrin. Increases of *Bifidobacterium* in the faecal flora were obtained by supplementing infant formula with bovine lactoferrin at 1 mg/mL (Kawaguchi *et al*., 1989a, b; Chierici *et al*., 1992; Roberts *et al*., 1992; Chierici, 2001), which suggests that feeding lactoferrin-enriched formula can lead to *Bifidobacterium*-dominant intestinal flora in infants. Current commercial applications of bovine lactoferrin include infant formulae, nutritional iron supplements and drinks, fermented milk, chewing gums, immune-enhancing nutraceuticals, cosmetic formulae and pet care supplements. Bovine lactoferrin as a food ingredient is thought to be safe because there is a long dietary history of its use, while buffalo lactoferrin use can be exploited further. Use of bovine lactoferrin as a nutritional supplement is considered to be GRAS (Generally Recognised as Safe) by the US Food and Drug Administration. Lactoferrin is used to supplement foods such as infant formula, supplemental tablets, yoghurt, drinks and sports foods, and skin and oral care products (Tomita *et al*., 2002; Wakabayashi *et al*., 2003).

24.2.2.3.3 Immunoglobulins

The function of immunoglobulins in colostrum and milk is to protect the neonatal calf and the mammary glands against pathogens (Lilius & Marnila, 2001; Elfstrand *et al*., 2002). The imunoglobulin content decreases sharply after the initial 2 days of lactation. Commercially, immunoglobulin products are used effectively in human and animal health care (Loimaranta *et al*., 1997, 1999; Korhonen *et al*., 2000; Casswall *et al*., 2002; Marnila & Korhonen, 2002; Marnila *et al*., 2003; Earnest *et al*., 2005). Immunoglobulins in buffalo colostrum, milk and blood were investigated by Mahran *et al*. (1997). Three classes of immunoglobulins have been identified: IgG, IgM and IgA. Satija *et al*. (1979) also detected immunoelectrophoretic patterns of IgG, IgA and IgM in buffalo colostrum. They observed cross-reactions of buffalo serum with seminal plasma, saliva and milk whey only in the IgG region. Using PAGE, lipoprotein (5.2% ± 0.41), IgM (11.4% ± 3.1), IgG (9.4% ± 0.98), haptoglobin (21.8% ± 3.73), transferrin (10.4% ± 2.15), ceruloplasmin (7.8% ± 1.3), postalbumin (20.8% ± 2.09) and albumin (13.7% ± 0.75) were identified. The IgG was present in two subclasses, IgG1 and IgG2.

Colostrum immunoglobulins contain higher values of all essential amino acids except Leu and Lys, and more non-essential amino acids than the corresponding serum. Goel and Kakker (1997) reported, from colostrum samples of three buffaloes, immediate postpartum values for IgG1 of 31.6, IgG2 2.0, IgM 33.6 and IgA 0.4 mg/mL, these levels declining sevenfold by 24 hours and more than 30-fold by 84 hours after parturition. Nawar (1999) purified immunoglobulins from buffalo serum and colostrum for their optimum recovery. Periodic changes in the chemical composition and amino acid content, and effects of heat treatment and soft cheesemaking on buffalo immunoglobulin content have also been studied (Mahran *et al*., 1997). Commercial pasteurisation results in incomplete denaturation of IgG1 and IgG2 in buffalo milk. El-Loly *et al*. (2007) studied denaturation of immunoglobulins in buffalo milk and reported that IgG and IgM are incompletely denatured on heating up to 88°C for 15 min whereas IgA is completely denatured at any temperature between 63 and 88°C. This indicates that IgA is the most heat sensitive of the immunoglobulins. The rate of denaturation of buffalo milk immunoglobulins is lower at 63°C compared with 88°C. El-Agamy (2000) found that the whole activity of IgG in

buffalo or cow milk was lost at 75°C per 30 min versus 69% loss of camel IgG. The concentrations of IgG, IgM and insulin-like growth factor-1 decreased by 97.9, 97.5 and 96.2% for buffalo and 77.0, 74.9 and 76.0% for cow colostrum, respectively, 5 days after parturition (Abd El-Fattah *et al*., 2012).

24.2.3 Carbohydrates

Buffalo milk has higher levels of lactose than cow, goat and camel milk. Carbohydrates are the primary source of energy for activity and glucose is the only form of energy that can be used by the brain. Excess glucose is stored in the form of glycogen in the muscles and liver for later use. Carbohydrates are important in hormonal regulation in the body. Lack of adequate levels of glucose in the blood and carbohydrate stores leads to muscle fatigue and lack of concentration. Lactose is a disaccharide made up of glucose and galactose bonded together. Before it can be used by the body, the bond must be broken by the enzyme lactase in the small intestine. People who have decreased activity of lactase in the small intestine may have problems digesting lactose and this is referred to as lactose intolerance or malabsorption.

24.2.3.1 Oligosaccharides

Milk oligosaccharides contribute to the growth of beneficial intestinal microflora, postnatal stimulation of the immune system and provide defence against bacterial and viral infections by acting as competitive inhibitors for binding sites on the intestinal epithelial surface (Kunz & Rudloff, 2002). Compared with human milk and colostrum, the levels of oligosaccharides in cow, sheep and goat milk are much lower (Urashima *et al*., 1997; Martinez-Ferez *et al*., 2006), whereas they are similar in buffalo milk. Currently, there are only limited data and research findings on oligosaccharides in buffalo milk. Abd El-Fattah *et al*. (2012) found that, at calving, all components decrease gradually as the transition period advances except lactose, which conversely increases. On the fifth day after parturition, the concentration of total protein, whey proteins, fat, ash and total solids decreases by 69.4, 91.5, 36.9, 45.6 and 43.9% for buffalo and by 76.0, 94.1, 53.4, 33.6 and 52.3% for cow colostrum. However, lactose concentration increases by 42.5% for buffalo and 57.4% for cow colostrum. The low concentration of oligosaccharides in cow milk and colostrum has stalled their utilisation as biologically active ingredients in the healthcare and food sector but it opens the door for milk and colostrum from buffaloes, which have comparable oligosaccharide levels as human milk. Much research interest has been shown recently on the potential of milk oligosaccharides in infant nutrition.

Milk oligosaccharides are divided into neutral and acidic classes (Gopal & Gill, 2000). The former do not contain any charged monosaccharide residues whereas the latter contain one or more residues of sialic acid that are negatively charged. About 150 and 200 oligosaccharides have been isolated from human and domestic farm animal milk and colostrum and their chemical structures determined (Boehm & Stahl, 2003; Nakamura & Urashima, 2004).

Buffalo milk is richer in oligosaccharides having immunostimulant activity, but so far isolation of oligosaccharides has not been reported from buffalo milk. Saksena *et al*. (1999) found that a processed oligosaccharide mixture of buffalo milk induced significant stimulation of antibody delayed-type hypersensitivity responses and stimulation of non-specific immune responses measured in terms of macrophage migration index. The authors isolated a novel pentasaccharide from the immunostimulant oligosaccharide fraction of buffalo milk with structure GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)Glc. Colostrum contains a complex mixture of oligosaccharides in the free form (Blanc, 1981). These oligosaccharides are of considerable interest because of their ability to enhance growth of *Bifidobacterium* (Gyorgy *et al*., 1974), serving as substrate for glycosyltransferases and hydrolases and as inhibitors of infectious agents of the neonatal gut (Gyorgy *et al*., 1974; Parkkinen & Finne, 1985), and because of their role in the development of immunity in newborns. Aparna and Salimath (1995) reported the composition of oligosaccharides, isolation and structural elucidation of disialyl lactose from the colostrum of buffaloes as three fractions: the first fraction contained glycopeptides (0.2–0.8%), while the second and third contained oligosaccharides (0.3–1.5% and 2.2–2.8%, respectively).

24.2.3.2 Minor sugar fractions

The galactose, *N*-acetylgalactosamine and sialic acid contents of buffalo κ-casein fractions ranged from 0 to 4.3, 5.5 and 8.5 mol/mol protein, respectively (Addeo *et al*., 1977). The maxima are higher than the corresponding figures in cow κ-casein fractions for sialic acid and galactosamine, and lower for galactose, with values ranging from 0 to 6.7, 3.5 and 4.3 mol/mol protein, respectively, for seven fractions isolated from cow κ-casein A (McKinlay & Wake, 1965). Gyorgy *et al*. (1974) discussed the protective effect of sialic acid bound to glycoproteins and oligosaccharides against bacterial degradation. Compositional analysis of the glycans revealed the presence of L-fucose, D-galactose, D-mannose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and *N*-acetylneuraminic acid in different proportions. A sialoglycopeptide was isolated from buffalo colostrum in pure form. It consisted of fucose, galactose, mannose, *N*-acetylglucosamine and *N*-acetylneuraminic

acid in the ratio 1 : 2 : 3 : 4 : 1; aspartic acid, serine, threonine, proline and glutamic acid were the major amino acids. Glycine was identified as the N-terminal amino acid residue. Oligosaccharides from human milk are considered as growth-promoting factors for the *B. bifidus* flora in the gastrointestinal tract of breast-fed infants. In the intestine, they act as anti-adhesive and anti-infective factors (Kunz & Rudloff, 2002). Aparna and Salimath (1996) observed the occurrence of *N*-acetylglucosamine 6-phosphate as an integral component in complex carbohydrates. Aparna and Salimath (1999) isolated acidic glycoproteins from buffalo colostrum at pH 4.6 and their yields were the highest in first-day colostrum, but decreased drastically in the samples from the third to fifth day.

24.2.4 Minerals

The mineral content of buffalo milk comprise a small proportion (8–9 g/L) in the form of ions which are more or less associated among themselves and with the proteins. These elements are not constant and are highly influenced by stage of lactation, nutritional and health status of the animal, environmental and genetic factors. The chemical form in which a macroelement or trace element is found in milk or in any food and supplement is very important. Its chemical nature will define its degree of absorption, utilisation, transport, cellular assimilation, conversion into biologically active form and thus bioavailability within the human body. Minerals have many roles in the body including enzyme functions, bone formation, maintenance of water balance, and oxygen transport. Mineral elements perform two major functions in the human body: construction (skeleton and all soft tissues) and regulation (associated with a variety of body systems: heartbeat, clotting of blood, maintenance of internal pressure of body fluids, nerve responses, transport of oxygen from lungs to tissues).

24.2.4.1 Major minerals

Buffalo milk contains more minerals than cow milk (Sahai, 1996; Ahmad *et al*., 2008a, b; Ahmad, 2010), particularly calcium, magnesium, inorganic phosphate and citrate. Schmidt and Payens (1976) reported that milk casein micelles are mainly composed of casein (93–94%) but in addition contain a small but essential amount of inorganic constituents (6–7%), mostly referred to as colloidal calcium phosphate (CCP), although magnesium and some citrate are incorporated in casein micelles as well. The physical state and stability of casein micelles is mostly dependent on the nature of these minerals, particularly CCP, which is affected by changing physicochemical conditions of the milk such as pH, ionic strength and heat treatments. Depending on the type of ions, they are soluble or partially associated with casein molecules in the colloidal portion. Total, diffusible and colloidal concentrations of the major minerals are shown in Table 24.3. As in cow milk, macroelements are differently distributed into diffusible and non-diffusible fractions in buffalo milk.

The total calcium and inorganic phosphate concentrations are higher in buffalo milk than in cow milk (Ahmad *et al*., 2008a, b). Ranjan *et al*. (2005) also observed this difference in total calcium concentration between milks of these species. Ahmad *et al*. (2008a, b) found that 82% and 72% of calcium and 66% and 48% of inorganic phosphate were in the micellar phases of buffalo and cow milk, respectively. These differences in micellar mineralisation (more mineralised casein micelles of buffalo milk) can be attributed to the higher phosphorylation of casein molecules in the case of buffalo milk or to a difference in the quantity of CCP. The micelles are broken down into casein molecules on removal of the inorganic constituents (Downey & Murphy, 1970), and micelles are formed by connecting subunits with calcium and inorganic phosphate. Varindra *et al*. (1994) found colloidal calcium at 1088 mg/kg and inorganic phosphate at 816 mg/kg in buffalo skimmed milk. Compositional analysis of buffalo milk micelles by Ganguli (1973) and Ahmad *et al*. (2009) disclosed that bound calcium and inorganic phosphate levels in buffalo casein micelles are higher than in cow casein micelles. Calcium and inorganic phosphate are mostly known as milk minerals and are associated with bone health.

24.2.4.2 Trace elements

Trace elements such as boron, cobalt, copper, iron, manganese, sulphur and zinc are present in buffalo milk (Table 24.3). Boron is a dynamic trace element in human, animal and plant nutrition (Nielsen, 1997). Boron is much more than just another mineral as it affects a broad range of life processes involving macrominerals, energy substrates such as glucose and triglycerides, amino acids and proteins, free radicals, bone mineralisation, prostate health, mental function, oestrogen metabolism and numerous body systems. Boron is a mineral that is critical to human health. One of the first recognised roles of boron in human nutrition was its contribution to promoting and maintaining good bone mineralisation.

Cobalt is similarly present in buffalo and cow milk. It is a key to promoting bacteria in the rumen that is vital to the formation of vitamin B_{12}. This process is also vital in the breakdown of propionic acid as a major ruminant energy source and helps in the transport of folic acid to the liver through the formation of methionine.

Copper is a component of the enzymes used in iron metabolism. It helps to form red blood cells and is important in the construction of hair and muscle. It is also helpful to the utilisation and proper metabolism of proteins.

Iron is a component of blood and many enzymes. It is involved in blood metabolism and oxygen transport. Milk contains a small amount of iron but its concentration in buffalo milk is higher than that in cow milk (Table 24.3).

Manganese controls energy production by controlling sugar levels and is important for bone formation, protein metabolism and muscle regulation. It is a key element for conception.

Zinc helps carry carbon dioxide to the lungs, fight infection and keeps skin and hair healthy. It is important in both male and female fertility. Zinc is a component of many enzymes and proteins, and is involved in gene regulation. For all these physiological functions in the human body, buffalo milk can be considered a good source.

Dobrzañski *et al*. (2005) studied 38 microelements and trace elements in raw cow milk and found that the location of cows has a significant impact on the content of these elements in milk. In another study, infant formula samples sold in Nigeria, the UK and the USA were analysed for various essential elements (Ca, Co, Cu, Cr, Fe, Mg, Mn, Mo, Na and Zn) and non-essential elements (Ag, Al, As, Ba, Be, Cd, Hg, Ni, Pb, Sb, Sn, Sr, Ti, Tl, U and V) (Ikem *et al*., 2002). They found that soy-based powder infant formulae generally had higher element levels than milk-based powder formulae, and that some infant formula powder had low nutritional contents compared with the recommended daily allowance (RDA) and dietary reference intakes. Imran *et al*. (2008) studied different buffalo milk and mixed industrial milk samples in Pakistan and found that sodium, potassium, calcium and magnesium were below the standards in commercially available milk in Pakistan markets to meet World Health Organization requirements.

24.2.5 Enzymes

Milk contains numerous minor proteins found mainly in the whey and milk fat globule membrane fractions. These minor proteins do not have significant functional properties like casein and whey fractions, but many have been identified as having physiological effects. The minor proteins include enzymes, metal-binding proteins, enzyme inhibitors, vitamin-binding proteins and numerous growth factors (Fox, 2001). Several minor dairy proteins have been included as bioactive ingredients in nutraceutical products.

24.2.5.1 Lysozyme

Lysozyme, a low-molecular-weight basic protein enzyme, is an important component of the antibacterial system in milk. Together with lactoferrin, lysozyme is one of the most extensively studied antibacterial milk proteins. The sequence of 23 amino acid residues at the *N*-terminal end of buffalo milk lysozyme shares 57% homology with cow milk lysozyme, 48% homology with human milk lysozyme, 35% homology with egg-white lysozyme and 30% homology with equine milk lysozyme. Priyadarshini and Kansal (2002b) found the molecular mass of buffalo milk lysozyme to be 16 kDa compared with 14.3 kDa for standard egg-white lysozyme. Milk lysozyme from different species is antigenically different. Egg-white lysozyme shows no cross-reactivity with anti-buffalo milk lysozyme. Antibacterial activity of lysozyme from buffalo milk and egg white has been compared by Priyadarshini and Kansal (2002b). Lys at position 1, Cys at 6, Ala at 9, Asp at 18, and Gly at 16 and 22 are conserved in all five lysozymes, while positions 3 and 20 have aromatic amino acids (Phe or Tyr) and positions 1 and 5 have basic amino acids (Lys or Arg) in all five lysozymes.

Important variations in buffalo milk lysozyme are Arg at position 4, Ile at 13, Asn at 7 and Ala at 8, 17 and 19, which differ from the other four lysozymes (Priyadarshini & Kansal 2002a, b). At the practical level, lysozyme has found application in food preservation, and egg white lysozyme is already used successfully as an antimicrobial in many foods, especially cheese (Benkerroum, 2008). Buffalo colostrum contains five times more lysozyme activity than mature milk (Priyadarshini & Kansal 2002a) and the specific activity of buffalo milk lysozyme ($60 \pm 3.9 \times 10^{-3}$ units/mL) is higher than that of cow milk lysozyme ($29.1 \pm 1.5 \times 10^{-3}$ units/mL). Buffalo milk lysozyme is active over a wide range of pH values and its activity is strongly influenced by the molarity of the medium. Lysozyme in buffalo milk is more stable than in cow milk during storage and heat treatment (Priyadarshini & Kansal, 2002b). Lysozyme is a major component of the whey fraction in human milk (0.4 g/L). Human and buffalo milk lysozyme possess greater positive charges than egg white lysozyme and are about three times more active. Lysozyme activity in buffalo milk is not influenced by parity and stage of lactation; however, it increases during extreme weather conditions in winter and summer. Lysozyme in buffalo and cow milk exhibits maximum activity at pH 7.4. In another study, lysozyme activity was determined in milk from normal cows and buffaloes (somatic cell count $<5 \times 10^5$ cells/mL milk), and in buffalo colostrum. In cow milk, lysozyme activity ranged from 17.8×10^{-3} to 38.2×10^{-3} units/mL. The average lysozyme activity in cow milk (2.92 units/dL) is equivalent to 32 µg of standard egg white lysozyme (Chandan *et al*., 1965; Panif-Kuncewiez & Kisza, 1976; Van Nieuwenhove *et al*., 2004).

Lysozyme activity in milk from normal buffaloes ranged from 37.3×10^{-3} to 73.4×10^{-3} units/mL, which is higher than that reported for milk from cows (Chandan *et al*., 1965; Panif-Kuncewiez & Kisza, 1976), goats (Chandan *et al*., 1965), sheep (Chandan *et al*., 1965) and camels

(El-Agamy *et al*., 1992). The higher lysozyme activity in buffalo milk is possibly one of the factors responsible for lesser incidences of udder infections in buffaloes. The activity of lysozyme in buffalo colostrum (301.7×10^{-3} units/mL) is about five times the activity observed in mature buffalo milk (60×10^{-3} units/mL). In human (Chandan *et al*., 1964) and bovine (Panif-Kuncewiez & Kisza, 1976) milk and colostrum, lysozyme is in similar concentrations. A buffalo calf receives greater amounts of lysozyme during the first few days after birth, which might play an important role in prevention of enteric infections (Priyadarshini & Kansal, 2003). Buffalo milk lysozyme is fully stable (El-Dakhakhny, 1995; Priyadarshini & Kansal, 2002a), whereas cow milk lysozyme is partly inactivated by pasteurisation; however, Van Nieuwenhove *et al*. (2004) found that lysozyme in Murrah buffalo milk is completely inactivated by low (65°C per 30 min) and high (72°C per 15 s) pasteurisation conditions.

Lysozyme in buffalo milk is more stable than in cow milk during storage. An assay of lysozyme activity in milk can be used to diagnose mastitis in cattle, but not in buffalo. Some buffaloes exhibited a 1000-fold greater lysozyme activity and moderately raised somatic cell counts in milk, but there was no sign of mastitis (Priyadarshini & Kansal, 2002a). El-Agamy (2000) reported that loss of activity of lysozyme at 85°C per 30 min was 56%, 74% and 82%, respectively, in camel, cow and buffalo milk. Buffalo milk is relatively more resistant to microbial spoilage than cow milk. The favourable ionic environment of buffalo milk and the high specific activity of lysozyme can play important roles in preventing growth of some Gram-positive microorganisms. Lysozyme is an important antimicrobial agent in milk, and kills bacteria by cleaving the $\beta(1\rightarrow4)$-glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine residues of the peptidoglycan in the bacterial cell wall. The antibacterial activity of buffalo milk lysozyme against microorganisms has been found to be very effective: four Gram-positive bacteria were inhibited, while Gram-negative bacteria were resistant (Priyadarshini & Kansal, 2002b). El-Dakhakhny (1995) investigated effects of heat treatments of 60–90°C for 5–30 min on lysozyme activity in buffalo milk. The concentration of lysozyme was higher in buffalo milk (29 µg/dL) than in cow milk (21 µg/dL).

24.2.5.2 Lactoperoxidase

Lactoperoxidase is an enzyme present in milk of different species in varying concentrations and which has antimicrobial properties. Lactoperoxidase is also present and active in other secretory fluids of the body (Clare *et al*., 2003). Buffalo milk lactoperoxidase has been studied extensively (Kumar & Bhatia, 1994, 1998, 1999; Kumar *et al*., 1995; Van Nieuwenhove *et al*., 2004). Ozdemir *et al*. (2002) purified water buffalo lactoperoxidase from skimmed milk and found, at optimum pH and optimum temperature, the K_m to be 0.82 mmol/L and the V_{max} to be 13.7 µmol/mL per min. Kumar and Bhatia (1999) observed that lactoperoxidase is more stable in whey prepared from buffalo milk, with the relative order of thermostability being neutralised acid whey > rennet whey > skimmed milk. The Arrhenius energy values for buffalo skimmed milk, rennet whey and neutralised acid whey were 710, 1022 and 1398 kJ/mol, respectively. Buffalo lactoperoxidase is pH sensitive and undergoes denaturation at low pH, but is relatively stable in the range pH 5–10 (Kumar & Bhatia, 1994). Sato *et al*. (1996) reported greater heat stability of lactoperoxidase toward acidic pH in cow milk. Kumar and Bhatia (1999) reported that at 72°C buffalo milk lactoperoxidase alone in acetate buffer (0.1 mol/L, pH 6.0) was completely inactivated at zero time, while the presence of salts induced thermal protection of lactoperoxidase structure. The relative effect was in the order $KCl > NaCl > CaCl_2 > MgCl_2$, but sulphates of sodium, potassium and magnesium had no thermoprotective effects.

Lactoperoxidase is the most abundant enzyme in buffalo milk. Because of its broad biocidal and biostatic activity, lactoperoxidase has found many commercial applications, especially targeting oral pathogens (Tenovuo, 2002). Peroxidase activity of buffalo milk is normally two to four times higher than that of cow milk. Because of high peroxidase activity, buffalo milk can be preserved naturally for a longer period.

24.2.5.3 Xanthine oxidase

Gandhi and Ahuja (1979) determined the xanthine oxidase content of milk from cows, goats and buffaloes and found the highest levels in cows, which was negatively correlated with the fat content of the milk. In buffaloes, xanthine oxidase activity is increased in colostrum and then decreases with the change to normal milk. The activity of partially purified xanthine oxidase from buffalo milk fat globules was optimal at pH 7.6. The K_m and V_{max} values with xanthine as substrate were 48–55 µmol/L and 92–125 µmol/mg per min protein, respectively. In the presence of phospholipids, especially phosphatidylserine and phosphatidylinositol, the temperature-dependent inactivation of xanthine oxidase is decreased, indicating a protective effect of phospholipids on xanthine oxidase.

24.2.6 Vitamins

Buffalo milk is a rich source of fat-soluble and water-soluble vitamins. The average concentrations are given in Table 24.3. Vitamins have many roles in the human body

including metabolism cofactors, oxygen transport and antioxidants. They help the body to use carbohydrates, protein and fat.

24.2.6.1 Fat-soluble vitamins

Vitamin A content is higher in buffalo milk than in cow milk. The reason is that the carotenoid pigment, which is a precursor of vitamin A, is totally absent in this milk. It is a unique feature of buffalo milk which makes it whiter than other milks. Because of the absence of carotenoids and a higher fat concentration, total potency per unit weight of fat is lower in buffalo than in cow milk (Sahai, 1996). Vitamin A is a fat-soluble vitamin involved in vision, gene expression, reproduction and immune responses. At calving, the concentration of vitamin A in buffalo colostrum is approximately 1.5 times lower than in cow colostrum (Abd El-Fattah *et al*., 2012).

Vitamin D is a fat-soluble vitamin that is important in maintaining blood calcium and phosphorus balance and assists calcium metabolism. Buffalo milk is typically fortified with vitamin D and becomes a good source of vitamin D.

Vitamin E is a fat-soluble vitamin that has antioxidant activity. The compounds with vitamin E activity are the tocopherols and tocotrienols. Milk contains a small amount of vitamin E, which increases with increasing fat content of dairy products. Abd El-Fattah *et al*. (2012) found that buffalo colostrum has a higher concentration of vitamin E than cow colostrum. Tocopherol content is higher in buffalo milk than in cow milk (Table 24.3). It is even several times higher in buffalo colostrum. Milk is the most important source of retinol and α-tocopherol for calves. These antioxidants preserve the food quality and prevent lipid oxidation in the mammary gland and the calf's growing tissues. In buffalo milk, the levels of retinol and α-tocopherol are 2 and 1.7 times higher in winter than in summer, respectively (Spagnuolo *et al*. 2003).

Vitamin K is a fat-soluble vitamin involved in blood clotting, bone metabolism and protein synthesis. Buffalo milk contains a small amount of vitamin K, which increases with the fat content in dairy products.

24.2.6.2 Water-soluble vitamins

Buffalo milk thiamine is more stable to light than ascorbic acid and riboflavin. Mohammad *et al*. (1990) found that 33–34% thiamine was lost on exposure to light for 6 hours in the presence of oxygen, whereas riboflavin in buffalo milk appeared to be slightly more resistant to degradation on exposure to light as it degraded 77% versus 82% in cow milk on 1-hour exposure to direct sunlight (Sikka *et al*., 1990). Thiamin is a water-soluble vitamin that is an enzyme cofactor involved in the metabolism of carbohydrates and branched-chain amino acids. Riboflavin is a water-soluble vitamin that is an enzyme cofactor involved in electron transport reactions.

Small amounts of folate, pantothenic acid, vitamin B_6 and niacin are also found in buffalo milk. Folate is one of the water-soluble vitamins of the B group and is an enzyme cofactor important in the metabolism of proteins and nucleic acids and in blood functioning. Niacin is a water-soluble vitamin that is an enzyme cofactor involved in electron transport reactions required for energy metabolism. Pantothenic acid is a water-soluble vitamin that is an enzyme cofactor in fatty acid metabolism. B_6 is a water-soluble vitamin involved in the metabolism of proteins and glycogen (energy stored in the liver and muscles) and in the metabolism of sphingolipids in the nervous system.

Vitamin C is a water-soluble vitamin that is an important antioxidant. It has a role in collagen formation in connective tissue and helps in iron absorption and healing of wounds and injuries. There is a small amount of vitamin C in buffalo milk. Ascorbic acid in buffalo milk appeared to be more stable than in cow milk when heat treated and when exposed to sunlight. This characteristic was explained by Shekar and Bhatia (1985) as being due to the higher fat content of buffalo milk.

24.3 NUTRITIONAL AND HEALTH BENEFITS OF BUFFALO MILK AND ITS PRODUCTS

Buffalo milk is considered a superior quality raw material for processing and the manufacture of a large number of dairy products, Western and traditional or indigenous (Pandya & Khan, 2006). To the suckling young animal, milk not only provides nutrients but also myriad molecules that prime the neonatal mucosal immune system against bacterial infection. Milk contains natural bioactive substances (Chatterton *et al*., 2006) with strong interactions with human health. Increased awareness of the relationship between diet and health has brought about new trends in nutrition sciences, particularly to replace existing sources with better alternatives for resolving health disorders or nutritional issues, thanks to advances in analytical techniques and new technologies. The effects of buffalo milk on human health have been studied very little due to the presence of most of these animals in non-research-oriented regions of the world, but its nutritional importance cannot be neglected. According to recent research, whey proteins are considered to overcome blunted muscle response to nutritional stimuli and thus offset sarcopenia, the degenerative loss of skeletal muscle mass that affects older people (Yang *et al*., 2012).

24.3.1 Buffalo health

Before entering into a discussion of the nutritional and health benefits of buffalo milk, the importance of buffalo health itself must be considered, because the nutritional quality of milk depends on the animal's health. When compared with other domestic livestock, the water buffalo generally is a healthy animal. This is particularly impressive because most buffaloes live in hot humid regions that are conducive to diseases and they may be susceptible to most of the diseases and parasites that afflict cattle. Although the reasons are not specifically known, the effect of diseases on the buffalo and its productivity is often less deleterious than on cattle in the same ecosystem. Antibiotics and vaccines developed for cattle work equally well on buffaloes. As a result, treatments are available for most of the serious diseases of buffaloes, although some are not very effective for both.

The major cause of losses in buffaloes is calf mortality. Newborn buffalo calves, like cow calves, succumb in large numbers to viruses, bacteria and poor nutrition. This is largely due to poor management during the calf's first 2 months of life, especially when the calf is deprived of the dam's milk, and the proclivity of buffaloes for wallowing in water exposes calves to waterborne diseases. Further, a calf occasionally drowns when an adult rolls on top of it. In countries such as the Philippines, Vietnam, Cambodia, Laos, Malaysia, Thailand, Myanmar (Burma) and India, buffaloes, as reported by several workers, are highly susceptible to rinderpest, maybe even more so than indigenous cattle managed under similar conditions. In south-eastern Europe, however, the buffalo is relatively more resistant than Asian cattle breeds.

Buffaloes are less susceptible than cattle to foot and mouth disease; nevertheless, severe outbreaks among buffaloes are not uncommon. Water buffaloes are highly susceptible to pasteurellosis or haemorrhagic septicaemia caused by the bacterium *Pasteurella multocida*. Haemorrhagic septicaemia runs a more acute course in buffaloes than in cattle and the oedematous form is more common. Buffaloes are more susceptible to it than cattle and die in large numbers when affected by pasteurellosis. A vaccine against pasteurellosis is effective in protecting both buffaloes and cattle.

Reports on the incidence of anthrax in buffaloes vary from country to country. In Egypt the disease appears to be rare in buffaloes, and in India and Pakistan the disease is less common in buffaloes than in cattle. However, the situation is just the reverse in Myanmar, where buffaloes frequently contract the anthrax infection; this poses a serious danger to the elephants working in adjacent forests.

Several workers have reported that the buffalo is relatively less resistant to tuberculosis. Tuberculosis is rarely diagnosed in farm animals in the private sector, as farmers do not routinely test for this disease and the disease progresses unnoticed and untreated. On public sector farms, some routine screening tests are performed and the infected animals are culled. Animals testing positive are a public health issue, and animals are slaughtered after a positive diagnosis. Disposal of the milk is not normally needed, as the animal is slaughtered. Disease control and eradication programmes are not very organised or effective due to resource constraints on governments and the poor socio-economic status of farmers in countries such as India and Pakistan. This is one of the most important diseases affecting buffaloes in Egypt, the incidence being higher in housed buffaloes in the southern region. Slaughterhouse examinations of carcasses in a Cairo abattoir showed that about 7% of the buffaloes slaughtered had tuberculous lesions. The prevalence of tuberculosis in buffaloes is higher in old animals (6–8 years or more), and is 2.5–8.5% in Pakistan (Javed *et al*., 2006) and sometimes even more than 10% (Khan *et al*., 2008), with a significant decrease in milk production. Factors controlling the prevalence of this disease include the number of animals per farm, older age of animals, high milk production and parity, rather than the conditions on the farm (Javed *et al*., 2006; Khan *et al*., 2008). Lall *et al*. (1969) found that the incidence of tuberculosis reactors is higher in buffaloes than in cattle. Tuberculosis occurs among buffalo herds worldwide because most of them are kept in unsanitary conditions. It is a serious health concern, with no control of milk collection and no quarantine measures for buffalo, and can be a major source of infection for infants and adult consumers (Khan *et al*., 2008).

Studies in buffaloes have revealed that the serological prevalence of brucellosis is 3.3–26.1% (Ajmal *et al*., 1989; Akhtar *et al*., 1990; Ahmad & Munir, 1995). The incidence is higher in buffaloes maintained on organised farms compared with smallholdings (Ahmed *et al*., 1990). No official policy of brucellosis eradication exists in buffalo-rearing countries. Veterinary services, economic conditions and methods of farming in these countries suggest that the appropriate method for the control of brucellosis is immunoprophylaxis, although vaccines against brucellosis are not manufactured (Afzal *et al*., 2000). Brucellosis is present in buffalo-rearing countries, with a high percentage of abortions in positive-testing animals. The animals are usually tested for the disease after abortion. However, the milk is still used for public sale. There is no large-scale programme for screening and eradication of this disease. It is also a public health problem as the butchers, farmers, veterinarians, artificial insemination technicians and milk dealers come in contact with aborted tissue and infected animals. These people and their families are at high risk of

infection and display undulating fever, which lasts a long time and does not respond well to routine treatments.

Mastitis is one of the serious diseases of buffaloes, especially in countries where they are mainly kept for milk production, like India and Pakistan. A study showed an incidence of about 21% for subclinical or insidious cases, and 2.4% for clinical cases of mastitis in buffaloes in India. In Egypt, the incidence of mastitis in buffaloes and cows was virtually the same (Wahby & Hilmy, 1946). Mukherjee (2008) studied antioxidant, anti-inflammatory and phagocytic activities in milk polymorphonuclear cells isolated from healthy and mastitic buffaloes (and treated with vitamin E plus selenium). Somatic cell count decreased significantly in milk from buffaloes given this treatment. The anti-inflammatory activity was also higher in the treated animals; moreover, in milk of treated animals the phagocytic activity (percentage of neutrophils that had phagocytosed one to six bacteria) and phagocytic index (average number of bacteria/leukocytes counted in 100 cells) also increased significantly. Thus vitamin E plus selenium have health beneficial characteristics not only for humans but also for diseased animals.

Tick-borne parasitic diseases, for example theileriosis, babesiosis, anaplasmosis and trypanosomiasis, induce paralysis or toxicosis and cause physical damage and are considered a major obstacle in health and dairy performance of cattle and buffaloes. The prevalence of babesiosis in Pakistan has been reported by different workers as 5.5–42.8% in cattle and buffaloes, variations depending on factors like age, breed, season and tick activity (Rajput *et al*., 2005).

In India and Pakistan, exogenous oxytocin is being used by farmers to increase milk production. Oxytocin is usually injected intramuscularly at a dose of 10–20 IU immediately before each milking. Indiscriminate use of oxytocin without veterinary advice has resulted in loss of productive and reproductive performance of these animals (Mustafa *et al*., 2008).

Advanced research in buffaloes is lacking with regard to incidence, mode of transmission and economic losses for zoonotic milk-borne diseases such as anthrax, cholera, diphtheria, dysentery, foot and mouth disease, gastroenteritis, mastitis, milk sickness, paratyphoid fever, scarlet fever, septic sore throat, smallpox, tuberculosis, typhoid fever and brucellosis/undulant fever. These diseases can cause serious illness in humans but the incidence in various age groups and its association with body mass index, exposure to infected animals and people, and the loss of productive life of patients needs to be documented. The resistance of animals to diseases needs to be studied in local, exotic and upgraded cattle breeds. The tuberculin test and polymerase chain reaction techniques are used in diagnosis. These diseases have significant effects on the economics of dairy stakeholders and on the nutritional composition of buffalo milk. The direct and indirect contributors to financial losses are primarily loss of milk production, followed by purchase of additional feed, culling of buffaloes, labour inputs, veterinary fees, transport, deaths, drugs, abortions and chemicals. Because of informal methods of management, lack of record-keeping for disease outbreaks, high dependency on imported vaccines and lack of implementation of regulations for control of these diseases in buffalo-rearing countries, nutritional deficiency disorders and health problems are common not only in the animals themselves but also in humans, and the major reason for this is lack of interest by national governments.

24.3.2 Effect of buffalo milk on particular diseases

The majority of buffaloes are managed by small farmers living in rural areas, and more than 60% of the milk is used for their household needs. For these families buffalo milk consumption is essential for maintaining good health and normal activities. The health benefits of milk include good bone health, smooth skin, strong immune system, and prevention of illnesses such as hypertension, dental decay, dehydration, respiratory problems, obesity, osteoporosis and even some forms of cancer.

24.3.2.1 Osteoporosis

Osteoporosis is a disease that is characterised by decreased bone mass and deterioration of the bone tissue leading to an increased risk of fracture. Bone is approximately 50% protein and 50% calcium phosphate. Bone serves as a reservoir for calcium in the body. Buffalo milk and fortified dairy products also provide vitamin D, which regulates calcium absorption into bones and other important components of bone metabolism such as phosphorus, protein, magnesium and zinc. Buffalo milk contains a larger amount of calcium per serving than other foods. According to the Institute of Medicine (1997), the RDA for calcium and phosphorus is 1000 and 700 mg/day, respectively, for those aged 19–50 years. A 200-mL serving of cow milk gives 24% and 17% of the RDA for calcium and inorganic phosphate, respectively, whereas the same serving of buffalo milk can provide more than 38% and 25% of RDA for both elements, respectively (Ahmad, 2010). Because of the high amount of calcium in buffalo milk, it can have great importance for controlling osteoporosis.

24.3.2.2 Allergy

People who suffer from cow milk allergy may find buffalo milk a good alternative. A study has revealed that a child with cow milk allergy was able to tolerate water buffalo milk (Sheehan & Phipatanakul, 2009). There are allergenic

proteins in cow milk that are not contained in buffalo milk, and vice versa. Cow milk can cause eczema in babies and small children, but they may be able to tolerate buffalo milk or buffalo milk products (BBC, 2005). Several case studies have indicated that permanent use of buffalo milk decreased the severity of eczema. A report by the BBC (2005) showed that buffalo milk is a healthy alternative to other milks. Proteomic techniques coupled to immunological methods may make it possible to select other milk products that do not contain the same allergens as ordinary cow milk. D'Auria *et al*. (2005) presented evidence that proteomic evaluation of proteins from different mammalian species including buffalo is a suitable method of testing whether proteins from the milk of different mammalian species may be used as a substitute for untreated bovine milk. Proteomic evaluation of milk from different mammalian species may not only be of help when recommending suitable feeding in cases of cow milk allergy but also gives new insight into the background to allergic reactions caused by milk proteins (D'Auria *et al*., 2005).

According to a very recent study by Hinz *et al*. (2012), several spots observed in two-dimensional electrophoretograms of bovine, caprine, buffalo, equine and camel milk could tentatively be identified as polypeptides arising from the enzymatic hydrolysis of caseins. The understanding gained from the proteomic comparison of these milks may be of relevance in terms of identifying sources of hypoallergenic alternatives to bovine milk. Detailed studies on the characteristics of milk proteins, allergens and their possible reactions with other constituents *in vivo* are necessary before recommending substitutes. Cross-reactivity between food allergens occurs when they share part of their amino acid sequence, or when their three-dimensional molecular structure causes them to have a similar capacity to bind specific antibodies. Restani *et al*. (2002) found that animal monoclonal antibodies specific for cow milk proteins can recognise the majority of milk proteins from other mammals bred in Mediterranean countries, i.e. sheep, goats and buffaloes. An area of heterogeneity between animal and human species in a critical amino acid sequence (epitope) of an allergen can determine the degree of immunogenic activity.

24.3.2.3 Dental caries

Dental caries (tooth decay) is a major public health problem that plagues all countries in the world. According to Aimutis (2004), tooth enamel is a polymeric substance consisting of crystalline calcium phosphate embedded in a protein matrix. Dental caries develops by acidic demineralisation (calcium and phosphorus solubilisation) of tooth enamel. Demineralisation occurs directly (by acidic food consumption) or indirectly (by fermentation products of dental plaque odontopathogenic bacteria growing on residual food particles between teeth or adhering to the plaque). Fermentation of sugars by bacteria in the mouth results in the formation of acids which then lower the pH (to 5.5) of the mouth and allow mineral loss from tooth enamel. Research efforts with milk-derived bioactive peptides have focused on the inhibition of cariogenic plaque-forming bacteria, inhibition of tooth enamel demineralisation and subsequent enamel remineralisation.

Caseinophosphopeptides (CPP) and glycomacropeptide (GMP) have been patented for use in common personal hygiene products to prevent dental caries. Research has shown CPP and GMP to be growth inhibitory to the cariogenic bacterium *Streptococcus mutans* and other species (Aimutis, 2004). Additionally, CPP forms nanoclusters with amorphous calcium phosphate at the tooth surface to provide a reservoir of calcium and phosphorus ions to maintain a state of supersaturation with respect to tooth enamel. This would buffer plaque pH and also provide ions for tooth enamel remineralisation. Glycosidic structures attached to GMP are important to numerous bioactive properties of the peptide including anticariogenicity. Like CPP, GMP has been shown to inhibit enamel demineralisation and promotes tooth enamel remineralisation. Buffalo milk rich in CPP is a good option for curing dental caries and a pitted tooth surface by its buffering effect on mouth pH that prevents the pH from dropping to conditions favourable for demineralisation. Consumption of milk products, particularly cheese, has been shown in research studies to have a protective effect against dental caries (Aimutis, 2004). Although the mechanism by which cheese protects against dental caries is still not clear, milk proteins, calcium and phosphorus all contribute to this effect.

24.3.2.4 Cancer

Milk also has components that can help fight bacterial and viral infections and even prevent tumour growth. Whey proteins have been shown to provide protection against the development of cancer when given orally in animal models. Yoshida *et al*. (1991) found that this might be due to sulphur-containing amino acids in whey protein that can bind mutagenic heterocyclic amines with carcinogenic properties. Recent studies have indicated that protein-derived peptides released in buffalo cheese acid whey exert a cytomodulatory effect in human epithelial colon cancer (Caco-2) cells (De Simone *et al*., 2011). Structural characterisation of the active peptide fraction of buffalo cheese whey and its application to H-Caco-2 cells *in vitro* demonstrated its ability to modulate the cell cycle (De Simone *et al*., 2011). Deocaris *et al*. (2001) investigated the presence of human multimeric ALA in fresh buffalo milk. A buffalo ALA was isolated and this killed all SkBr3 breast

carcinoma cells at a concentration of 8.0 mg/mL. The possible mechanism by which buffalo ALA exerts its antitumour activity is presumed to be a combination of early necrosis through loss of plasma membrane integrity within 8 hours after treatment and largely via apoptosis (programmed cell death). There are several components in milk that are protective against cancer: calcium, vitamin D, CLA, sphingomyelin and whey proteins. Calcium and vitamin D may reduce the risk of colon cancer. Calcium and vitamin D consumption have also been shown to have a protective effect against breast cancer in humans. The protective effect of calcium and vitamin D has been observed in numerous human studies in several countries (Daniells, 2010). Sphingomyelin and whey proteins have been shown to inhibit colon cancer cell growth in laboratory settings. CLA is a very effective inhibitor in breast cancer development, and inhibits skin, stomach and colon cancers.

24.3.3 Role of constituents of buffalo milk and products in human nutrition and health

A summary of the major and minor constituents/nutrients in buffalo milk with their nutritional and health benefits is given in Fig. 24.3. Buffalo milk gives a higher yield of energy (Soliman, 2005) than cow milk. More than 100 calories are derived from 100 g of buffalo milk compared with 70 calories from 100 g of cow milk. Animal bioassays have shown the protein efficiency ratio (PER) of buffalo milk proteins to be 2.74 and that of cow milk 2.49. Buffalo milk fat, carbohydrates and protein contribute 62%, 21% and 17%, respectively, to the energy total.

Buffalo milk produces thick and creamy dairy products suitable for the manufacture of traditional/indigenous milk products (see section 24.1.4). Buffalo milk is used like cow milk for making different industrial dairy products such as butter, butter oil (clarified butter or desi-ghee), soft and hard cheeses, condensed and/or evaporated milks, ice cream, yoghurt and buttermilk (lassi) (Ligda, 1998). It is also used for cheesemaking in southern Italy, traditionally for the manufacture of Mozzarella cheese (Mozzarella di Buffalo) (Spanghero & Susmel, 1996; Zicarelli, 2004b). To produce 1 kg of fresh cheese, a cheesemaker requires 8 kg of cow milk but only 5 kg of buffalo milk (Ligda, 1998). Buffalo milk yoghurts are naturally thick set without additional milk proteins or gelling agents. Functional buffalo milk products are today probably the most talked-about food groups in buffalo milk-producing countries like India, Pakistan, China, Egypt and Italy, and have gained great prominence thanks to the growing health and nutritional concerns of consumers. Many of the traditional buffalo milk products have inherent health factors and there is also great potential to make other nutraceutical principles (Patel, 2007). There are many health beneficial claims based on buffalo milk which have not yet been scientifically proven and need careful and serious study.

24.3.3.1 Fatty acids and glycerides

New research has identified individual fatty acids with specific biological activity. The structure of the fatty acids plays a major role in maintaining health. Fatty acids are very important for infant nutrition, particularly the medium-chain fatty acids (higher in buffalo milk), and are an energy source in ruminants (Gotoh *et al*., 2012). Ratios of triacylglycerol (TAG) positional isomers in buffalo cheese fat were analysed very recently by Gotoh *et al*. (2012). The ratio of β-PPC [the TAG consisting of two palmitic acids (P) and one capric acid (C), with the palmitic acid located at the β position] and β-PCP in human milk was different from that in buffalo milk fat, with more than half of the medium-chain fatty acids located at the β position, when other fats possessed them mainly at the α position. Palmitic acid was mainly located at the β position in human milk, while the location in buffalo cheese fat was mainly at the α position.

According to Bindal and Wadhwa (2003) goat, cow and buffalo ghee have striking differences in their fractionation and melting behaviour, fatty acid composition, including saturated and unsaturated glycerides, and lactone profiles. Buffalo ghee has the lowest level of liquid fraction (26%) compared with goat (69%) and cow ghee (31%), with the highest melting and softening points among these species. Buffalo ghee has less unsaturated glycerides (56%) than goat ghee (65%) but is similar to cow ghee (55%). The higher proportion (9–12%) of high melting triglycerides gives it a bigger grain size (fat), which in turn imparts a grainy texture to ghee obtained from buffalo milk. Apart from this, buffalo milk ghee is less prone to hydrological rancidity than cow milk ghee and has a greater shelf-life. These differences show the importance of utilising buffalo ghee or its liquid and solid fractions to formulate specific dairy products. The emulsifying capacity of buffalo milk fat is due to a higher proportion (50%) of butyric acid-containing triglycerides compared with only 37% in cow milk. This is the reason for higher yields of butter and ghee prepared from buffalo milk. The production of 1 kg of butter requires 14 kg of cow milk versus 10 kg of buffalo milk (Ligda, 1998; Menard *et al*., 2010). Senel (2011) analysed acetaldehyde, acetone, butanone-2, diacetyl and free fatty acids in Afyon Kaymagi and found that the amount of some carbonyl compounds and the free fatty acid composition were generally different from other dairy products. Free fatty acids were the main compounds contributing to the aroma and flavour, with acetone as the predominant carbonyl compound. Butyric, palmitic, stearic and oleic acids within the free fatty acid composition were the predominant free fatty acids in Afyon Kaymagi.

24.3.3.2 Conjugated linoleic acid

Recently, Van Nieuwenhove *et al*. (2011) determined the influence of buffalo dairy products on lipid content and CLA incorporation in the liver and intestine of mice. Mice fed buffalo cheese showed the highest gain in body weight. CLA was only detected in fat tissues of mice fed buffalo dairy products, with *cis*-9, *trans*-11 being the major isomer. The authors fed mice buffalo milk and buffalo Mozzarella cheese, and higher linolenic (C18:3) acid contents were found in their tissues than mice fed the control diet without buffalo milk and buffalo Mozzarella cheese, showing higher absorption of CLA from buffalo milk. Lipoperoxides (thiobarbituric acid-reactive substances) were lower in groups receiving buffalo milk or cheese. Overall, a protective effect of buffalo cheese and milk on intestinal cells was determined. In a previous study, Van Nieuwenhove *et al*. (2007) determined the ability of some tolerant bacteria to produce CLA from free linoleic acid, with percentage conversions between 17 and 36%. *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus* showed the highest linoleic acid conversion in buffalo milk supplemented with different concentrations of linoleic acid. At a linoleic acid concentration of 200 µg/mL the production of CLA was twofold or threefold higher in milk than in MRS broth. The strain most tolerant to linoleic acid was *L.casei*. *Lactobacillus rhamnosus* produced the maximum level of CLA at high linoleic acid concentrations (800 µg/mL). The selected bacteria may be considered as adjunct cultures to be included in dairy fermented products manufacture. Low concentrations of linoleic acid must be added to the medium to enhance CLA formation; the production of CLA by bacterial strains using milk from regional farms as medium offers a possible mechanism to enhance this beneficial compound in dairy products and the possibility of developing functional foods.

24.3.3.3 Minerals

Eating calcium-rich, low-fat dairy products (such as buffalo milk and yoghurt) can boost the human body's fat-burning mechanism and reduce weight gain (Thomas *et al*., 2012). At high concentrations, calcium forms insoluble soaps with bile acids and fatty acids, neutralising their effect of stimulating proliferation of the intestinal mucous membrane. According to Barakat *et al*. (1969), buffalo milk contains more calcium and phosphorus and a higher calcium/phosphorus ratio (3.47) than cow milk (2.51) and less sodium and potassium than cow milk, which makes it a better nutritional supplement for infants (Malik, 2011). The role of calcium and phosphorus in supporting normal growth of skeleton and maintenance in later life has been well established. Average adults contain about 1250 g calcium, of which nearly 99% (1235 g) is stored in the skeleton and the remainder in teeth (7 g), soft tissues (7 g) and extracellular fluid (1 g). Calcium is combined with phosphorus as calcium phosphate in teeth and bones, and forms hard substances to provide rigidity. In a recent study, Feeney *et al*. (2002) observed the effects of higher concentrations of calcium on Mozzarella cheese quality and found that calcium concentration significantly affected the type and extent of proteolysis in Mozzarella cheese. In cheese with a 22 mg/g calcium-to-casein ratio, primary and secondary proteolysis were observed, whereas in cheese with a 29 mg/g calcium-to-casein ratio decreases in primary proteolysis were observed. Calcium plays an essential role in bone formation and metabolism, muscle contraction, nerve transmission and blood clotting. Dairy products are a significant source of calcium in the diet, and milk is the recommended source. Calcium from milk has three major advantages. It is particularly well absorbed and more bioavailable and most milk products contain significant quantities. Calcium bioavailability from milk and dairy products is about 30%, which is higher than in other foods and supplements, particularly plant-based ones (Weaver *et al*., 1991). Studies have shown that the interaction between calcium and other components in milk (P, vitamin D) confer specific health effects. Abrahamsen (2010) found that vitamin D given alone in doses of 10–20 µg is not effective in preventing bone fractures. By contrast, calcium and vitamin D given together reduce total bone fractures irrespective of age, sex or previous fractures. Phosphorus is involved in maintaining body pH, in storage and transfer of energy, and in nucleotide synthesis. Milk is a recommended source of phosphorus. Magnesium is an enzyme cofactor and is important in bone metabolism, and sodium is an electrolyte that is important in the maintenance of water balance and blood volume. Potassium is an electrolyte that is important in the maintenance of water balance, blood volume and blood pressure. Dairy products are a recommended source of potassium.

24.3.3.4 Bioactive peptides from caseins and whey proteins

Milk proteins provide excellent nutrition for the suckling animal and can also exert numerous physiological activities that benefit the young in a variety of ways, including enhancement of immune function, defence against pathogenic microorganisms and development of gut functions. Besides the naturally occurring biologically active proteins present in milk, a variety of nutritionally beneficial bioactive peptides are encrypted within the sequence of milk proteins that are released on suitable hydrolysis of the

precursor protein. Milk contains many protein components from which these bioactive peptides can be generated *in vivo* through gastrointestinal processes (Clare & Swaisgood, 2000; Korhonen & Pihlanto, 2006). The health benefits of dairy proteins for human consumption derive from (i) the intact whole protein; (ii) peptides of the partly hydrolysed protein; and (iii) the amino acids of the fully digested protein. Casein can be broken down into a number of smaller proteins (peptides) which have been shown to have specific benefits. Casein phosphopeptides can help prevent tooth decay by reducing demineralisation and promoting mineralisation by binding to dental plaque. They are also used commercially in oral care products and chewing gum. Angiotensin-converting enzyme (ACE) inhibitors are drugs that lower blood pressure. A number of naturally occurring peptides derived from casein have been shown to act as ACE inhibitors. Milk high in these peptides was shown to lower blood pressure in a study of people with high blood pressure. Minervini *et al*. (2003) studied peptide hydrolysates of sodium caseinates of bovine, sheep, goat, buffalo and human milk which were hydrolysed by a partially purified proteinase of *Lactobacillus helveticus* PR4. Various ACE-inhibitory peptides were found in the hydrolysates: the cow α_{s1}-casein 24–47 fragment (f), f169–193, and β-casein f58–76; sheep α_{s1}-casein f1–6 and α_{s2}-casein f182–185 and f186–188; goat β-casein f58–65 and α_{s2}-casein f182–187; buffalo β-casein f58–66; and a mixture of three tripeptides originating from human β-casein. The highest ACE-inhibitory activity of some peptides corresponded to an (*S*)-*N*-(1-[ethoxycarbonyl]-3-phenylpropyl)-Ala-Pro maleate (enalapril) concentration of 49.253 μg/mL (100 μmol/L). Several of the above sequences have features in common with other ACE-inhibitory peptides reported in the literature. These show a very large spectrum of inhibition against Gram-positive and Gram-negative bacteria, including species of potential clinical interest, such as *Enterococcus faecium*, *Bacillus megaterium*, *Escherichia coli*, *Listeria innocua*, *Salmonella* spp., *Yersinia enterocolitica* and *Staphylococcus aureus*. Petrilli *et al*. (1983) discovered that the most potent is the pentapeptide β-casein f60–64 opioid peptide, so-called β-casomorphin, in buffalo milk. Caseins are sources of antimicrobial peptides as well. The first discovery of the antimicrobial properties of milk was made by Jones and Simms (1930).

The antimicrobial activities of buffalo casein-derived peptides were studied by Aziz *et al*. (2004) and Bajaj *et al*. (2005). Buffalo casein was separated from pooled milk at pH 4.6 and subjected to hydrolysis by chymosin. The buffalo casein peptide fraction resulted in antimicrobial activity against *E. coli*, *Bacillus cereus* and *Kluyveromyces*. At a peptide concentration of 1000 μg/mL, a 50% reduction in the viable cell count of *E. coli* was observed; in *B. cereus*, the total count was reducted from 140×10^3 to 40×10^3 as the concentration increased to 1000 μg/mL. There was also a strong effect of peptides against yeast, with a 50% reduction at 250 μg/mL.

De Simone *et al*. (2009) discovered bioactive peptides in raw buffalo milk (Fig. 24.4) and waste whey of Mozzarella cheesemaking from buffalo milk. The mass spectrum showed the presence of two families of casein fragments, those deriving from the N-terminal end of α_{s1}-casein and those from the C-terminal end of β-casein. A multifunctional activity has already been described for the 193–209 region of bovine β-casein, which contains the bioactive sequence β-casokinin-10. Since the C-terminal sequence of β-casein is the same between river buffalo and cow species, precursors of peptides with immunostimulatory and ACE-inhibitory activities are therefore possibly also present in buffalo milk. Significant antiproliferative effect on Caco-2 cells was exerted by the peptides of buffalo milk mozzarella cheese whey.

A large amount of whey has to be disposed of yearly (80% milk volume remains as whey after transformation to Mozzarella cheese). Three kinds of buffalo whey are produced: sweet whey, formed immediately after or simultaneously to milk clotting; waste whey, the acid whey formed through curd maturation; and 'scotta', the liquid fraction remaining after removal of thermally coagulated whey proteins for the production of 'ricotta' from the sweet whey. After separation of the valuable whey proteins, the residue remaining is putrescible and has high chemical and biological oxygen demand. Buffalo waste whey contains large numbers of low molecular mass oligopeptides with potential bioactivity. These peptides exert greater reduction in H-Caco-2 cell proliferation and are absent in the peptide extract from raw buffalo milk, suggesting that the production of specific bioactive compounds occurs specifically during the production process of Mozzarella cheese.

The complexity of buffalo waste whey peptide extract makes it almost impossible to identify the peptide(s) responsible for the cytomodulatory activity. However, in buffalo waste whey, two peptides, β-casein f57–68 and f60–68, have been identified which are precursors of the agonist opioid β-casomorphin 7 and β-casomorphin 5 (Meisel & Schlimme, 1994).

It has been reported that casomorphin agonist peptides derived from the limited proteolysis of caseins, interacting with both opioid (Hatzglou *et al*., 1996a) and somatostatin receptors (Hatzglou *et al*., 1995), acted to decrease cell proliferation. In particular, Hatzglou *et al*. (1996b) have proved that in T47D human breast cancer cells *in vitro*, α- and β-casomorphins inhibit cell proliferation in a dose-dependent manner by interaction with δ- and κ-opioid receptors. Therefore, the proliferation decrease observed in

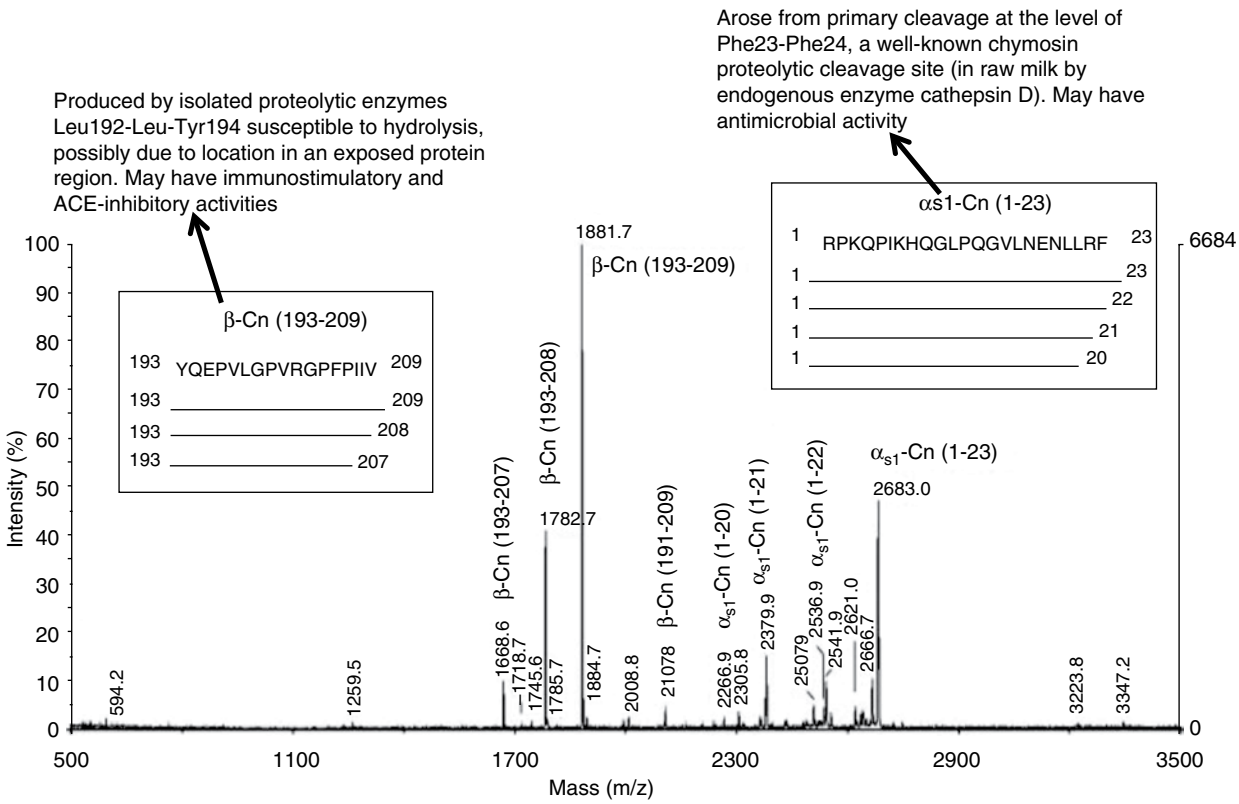


Figure 24.4. Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry of low molecular mass peptide fractions extracted from raw buffalo milk. Reproduced from De Simone *et al.* (2009), with permission of John Wiley & Sons.

colon cells could be mediated by a direct interaction between opioid precursors contained in buffalo waste whey peptide extract and the specific opioid and somatostatin receptors expressed on Caco-2 cells. The mild manufacturing conditions employed in the production of Mozzarella cheese could effectively preserve the bioactive molecules in whey, particularly in sweet whey and waste whey.

Surono *et al*. (2011) recently found a novel probiotic *Enterococcus faecium* (IS-27526) isolated from dadih (Indonesian traditional fermented buffalo milk) and investigated the effects of this bacterium on humoral immune response and on body weight of pre-school children. Total salivary sIgA and body weight increased significantly in children given probiotic compared with those given placebo. Changes in total salivary sIgA levels were significantly higher in underweight children supplemented with probiotic and significant weight gain was observed in children with normal body weight supplemented with probiotic. The novel probiotic *E. faecium* IS-27526 has significant positive effects on humoral immune response in underweight pre-school children and weight gain. According to Guo (2010), buffalo milk can be utilised for manufacturing a wide variety of dairy products. However, due to differences in compositional and physicochemical properties between both milks, processing technologies and equipment designed for cow milk are not directly suitable for buffalo milk processing, so modifications are necessary to gain more benefits from this milk through research.

24.4 CONCLUSIONS

The health aspects of buffalo milk are argued to be superior to those of cow milk, i.e. higher levels of fat, medium-chain fatty acids, saturated fatty acids, CLA, retinol and tocopherols, the unique presence of gangliosides, lower cholesterol content, higher protein, higher PER value, and higher mineral content, particularly calcium, iron and phosphorus. These characteristics may make this milk more popular in a health-conscious market. Buffalo milk's higher casein concentrations give better structural characteristics to lactic gels and better cheese yield. The presence of higher levels of various bioprotective factors makes it suitable for human nutrition and health, and it is a good

alternative for people suffering from cow milk allergy. Buffalo milk products and by-products may contain almost all the bioactive components of cow milk. Considerable scope is indicated for developing a range of dairy foods from buffalo milk with enhanced nutritional and probiotic attributes. The knowledge obtained so far will also drive research in the utilisation of industrial by-products from buffalo milk for the development of dietary supplements, nutraceutical foods, functional foods and novel drugs for the pharmaceutical industry.

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25
Sheep Milk

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25.1 INTRODUCTION

Although the production of sheep milk (about 8 million tonnes per year) is of marginal importance compared with cow milk in quantitative terms (2% of the total), nevertheless it is of major interest in Mediterranean and Middle Eastern countries where climatic conditions are not favourable for cattle raising. The numbers of sheep do not fully reflect the amount of milk produced, since they are often used for other purposes, such as meat and wool. Although sheep milk is richer in nutrients than cow milk, it is rarely used as milk for drinking. In general, sheep milk is utilised essentially for cheese production and in some countries is also made into yoghurt or whey cheeses (Haenlein & Wendorff, 2006; Mayer & Fiechter, 2012).

The nutritional importance of sheep milk is due to its composition (Table 25.1) since it generally contains higher total solids and major nutrient contents than goat and cow milk. As has been reported for bovine milk, the composition of sheep milk varies with diet, breed (and between individuals within a breed), parity, season, feeding and management conditions, environmental conditions, locality, and stage of lactation (Haenlein, 2001; Pulina *et al*., 2006). Sheep milk is an excellent source of high-quality protein, calcium, phosphorus and lipids. There is a good balance between the protein, fat and carbohydrate components, each being present in similar amounts. The supply of nutrients is high in relation to the calorie content. The fat and protein ratio is higher than in cow milk and therefore cheese yield is also higher (approximately 15% for sheep milk vs. 10% for cow milk).

Information on the nutritional characteristics of sheep milk is essential for successful development of dairy industries as well as marketing of their products. With progress in the knowledge of the composition and role of milk components, it has become apparent during recent years that some milk compounds possess biological properties beyond their nutritional significance and have an impact on body function or condition and ultimately on health. Major advances have occurred with regard to the science, technology and applications of these bioactive components present naturally in milk. These raw materials have proven to be a rich and unique source of chemically defined components that can be isolated and utilised as ingredients for health-promoting functional foods or as nutraceuticals. As a result, there is growing interest by the dairy industry in designing and formulating products that incorporate specific bioactive components derived from different kinds of milk. With the research tools available nowadays, the presence of many minor compounds with biological activity has been demonstrated in cow milk but less is known about ovine milk. The purpose of this chapter is to address the nutritional properties of sheep milk, mainly its lipids and proteins, with emphasis on the different bioactive compounds present in these fractions.

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Table 25.1. Composition of sheep and cow milk.

Component	Sheep			Cow
	Average content (%)	Range (%)	Average of dry matter (%)	Average content (%)
Water	81.6	79.3–83.3		87.3
Lactose	4.6	4.1–5.0	25.0	4.6
Fat	7.1	5.1–8.7	38.5	3.9
Crude protein (total nitrogen ×6.38)	5.7	4.8–6.6	31.1	3.2
Casein	4.4			2.6
Whey proteins	1.0			0.6
Non-protein nitrogen	0.1			0.1
Ash	0.9	0.7–1.1	4.9	0.8
Total solids	18.4	16.2–20.7		11.5
Non-fat solids	11.3			7.6

Source: adapted from Recio *et al*. (2009), with permission of Blackwell Publishing, and based on data from Walstra & Jenness (1984) and Ramos & Juárez (2011).

25.2 LIPIDS

Lipids are one of the most important components of milk in terms of physical and organoleptic characteristics, in addition to the nutritional properties that they confer on sheep dairy products. Furthermore, milk lipids are carriers of important vitamins as such as A, D, E, K and the carotenoids. Lipids are present in the form of globules, which in ovine milk are characteristically abundant in sizes of less than 3.5 µm. Among the ruminants, the average fat globule size is the smallest in sheep milk (Park *et al*., 2007). This is advantageous for digestibility and more efficient lipid metabolism compared with cow milk. The structure and composition of the globule membrane is similar to that of cow and goat milk fat and it represents approximately 1% of total milk fat volume (Lercker & Cocchi, 2010). Fat globules contain one hydrophobic lipid core, consisting mainly of triacylglycerides (TAGs), surrounded by a membrane comprising mainly phospholipids and glycoproteins. Among the health-beneficial components of the milk fat globule membrane (MFGM) are cholesterolaemia-lowering factor, inhibitors of cancer cell growth, vitamin binders, xanthine oxidase (bactericidal agent), butyrophilin (possible suppressor of multiple sclerosis), and phospholipids (agents active against colon cancer, gastrointestinal pathogens, Alzheimer's disease, depression and stress) (Spitsberg, 2005). All the above compel us to consider MFGM as a potential nutraceutical.

Different fatty acid compositions of the fat globule core and membrane have been reported in sheep milk (Scolozzi *et al*., 2006). The C14:0 and C16:0 fatty acids are present in greater amounts in the core, while polyunsaturated fatty acids (PUFAs) such as omega-3 fatty acids, conjugated linoleic acid (CLA) and precursors of the latter are better represented within the globule membrane. Thus the ratio of unsaturated to saturated fatty acids is lower in the fat globule core than in the membrane.

25.2.1 Triacylglycerides

TAGs constitute the largest group of lipids (~98%), including a large number of esterified fatty acids. TAGs in sheep milk exhibit a wide range of molecular weights according to the number of carbon atoms (taking into account the sum of the carbon atoms of the three acyl radicals) from C26 to C54 (Goudjil *et al*., 2003a; Fontecha *et al*., 2005; Mele *et al*., 2011). The TAG profile of sheep milk (Table 25.2) shows similarities to that reported for cow milk (Precht, 1992; Precht & Frede, 1994; MacGibbon & Taylor, 2006). However, sheep milk has a higher percentage of short-chain TAGs (C26–C34) than cow milk and a lower proportion of long-chain TAGs (C46–C52) (Goudjil *et al*., 2003a; Mele *et al*., 2011). Compared with TAGs containing mainly long-chain fatty acids, short-chain TAGs comprising saturated fatty acids with 6–10 carbon atoms have a lower melting point and smaller molecular size, are liquid at room temperature, and are less energy dense. These distinct chemical and physical properties affect the way they are absorbed and metabolised. Their medical and nutritional values have been the subject of research, demonstrating real benefits in different diseases (Haenlein, 2001). However, the unique content of about 25% C26–C36-chain TAGs in sheep milk fat and its possible quantitative modification through feeding has not been exploited commercially nor deeply explored in research. Given the great variance of fatty acids in milk fat, interest in the biological

Table 25.2. Triacylglyceride composition (mean values) of cow and sheep milk fat (wt%).

	Sheep		Cow	
Triacylglyceride (total acyl carbon number)	**Goudjil *et al.* (2003a)**	**Mele *et al.* (2011)**	**Precht & Frede (1994)**	**MacGibbon & Taylor (2006)**
C26	0.72	0.89	0.20	—
C28	1.60	1.88	0.60	0.60
C30	2.52	3.10	1.20	1.20
C32	3.63	4.66	2.60	2.50
C34	6.03	6.57	6.00	5.80
C36	9.64	9.39	10.90	11.00
C38	12.82	12.48	12.80	13.30
C40	11.98	12.60	10.10	10.70
C42	9.02	9.48	7.10	7.40
C44	8.08	7.95	6.70	6.70
C46	6.77	6.80	7.40	7.20
C48	6.67	5.38	9.10	8.60
C50	7.63	6.12	10.90	10.60
C52	8.43	6.81	9.50	9.40
C54	4.48	5.11	4.60	4.70

Source: based on data from the reference sources shown in the table.

effects of the position occupied by individual fatty acids in the TAG molecule on lipoprotein metabolism remains intense because of their relevant effects on cardiovascular disease (CVD). The fatty acid distribution in sheep TAG is non-random, with most of the C4:0 and C6:0 esterified to the *sn*-3 position and a high proportion of C16:0 occupying the *sn*-2 position, as in cow milk (Blasi *et al*., 2008). As pancreatic lipase in the gut selectively hydrolyses TAG at the *sn*-1 and *sn*-3 positions, short-chain free fatty acids and 2-palmitin monoglycerides are produced and absorbed. In contrast, free palmitic acid released from *sn*-1 and *sn*-3 positions may be lost as calcium-fatty acid soaps in the faeces. Therefore the predominant *sn*-2 position of C16:0 ensures that calcium in milk is highly absorbable (Lien *et al*., 1997; López-López *et al*., 2001).

25.2.2 Fatty acid composition

Milk fat is a complex mixture of about 400 different fatty acids comprising 4 to 26 carbon atoms, although only 30 of these compounds are present in a concentration above 0.1% while the rest are in trace amounts (Jensen, 2002). Saturated or unsaturated (with one to four double bonds) fatty acids mostly contain an even number of carbon atoms but there are also odd-numbered moieties (2%) as well as branched-chain fatty acids. From the quantitative viewpoint, the four major fatty acids in ruminants (C16:0, *cis*-9 C18:1, C14:0 and C18:0) account for more than 60% of the total (Table 25.3).

25.2.2.1 Saturated fatty acids

The content of saturated fatty acids (SFAs) in milk fat (~65%) has been associated with increments in CVD markers such as low-density lipoprotein (LDL)-cholesterol in human blood plasma. However, only C12:0, C14:0 and C16:0 seem to be unhealthy when consumed in excessive amounts (Legrand, 2008). Furthermore, research has revealed that it is more important to maintain a good balance among different fatty acids than to worry about the possible healthy or harmful effects they could exert individually (Parodi, 2004; Lecerf, 2008; Shingfield *et al*., 2008; Steijns, 2008). Other studies have concluded that those compounds considered hypercholesterolaemic may even have positive effects with moderate intake (Dabadie *et al*., 2005) and a recent meta-analysis of prospective epidemiological studies showed that there is no significant evidence for concluding that dietary saturated fat is associated with an increased risk of CVD (Siri-Tarino *et al*., 2010).

Sheep milk does not substantially differ in butyric acid (C4:0) content but does contain more caproic (C6:0), caprylic (C8:0) and capric (C10:0) acids than cow milk (Table 25.3). These fatty acids are associated with the characteristic flavour of cheeses and possess different biological properties. It has been reported that low concentrations of butyric acid can inhibit growth in a wide range of human cancer cell lines, including prostate and several other types of cancer (Williams *et al*., 2003; Blank-Porat *et al*., 2007). Animal studies have shown that dietary fibres,

Table 25.3. Mean values and minimum and maximum contents of sheep and cow milk fat main fatty acids (% in total fatty acid methyl esters).

	Sheep		Cow	
Fatty acid	**Mean**	**Min./Max.**	**Mean**	**Min./Max.**
C4:0	3.5	3.1–3.9	3.9	3.1–4.4
C6:0	2.9	2.7–3.4	2.5	1.8–2.7
C8:0	2.6	2.1–3.3	1.5	1.0–1.7
C10:0	7.8	5.5–9.7	3.2	2.2–3.8
C12:0	4.4	3.5–4.9	3.6	2.6–4.2
C13:0	0.2	0.1–0.2	0.2	
C14:0	10.4	9.9–10.7	11.1	9.1–11.9
iso C15:0	0.3	0.3–0.4	0.4	
anteiso C15:0	0.5	0.3–0.6	0.4	
C15:0	1.0	0.9–1.1	1.2	0.9–1.4
iso C16:0	0.2	0.2–0.3	0.4	
C16:0	25.9	22.5–28.2	27.9	23.6–31.4
iso C17:0	0.5	0.4–0.6	0.5	
anteiso C17:0	0.3	0.3–0.4	0.5	
C17:0	0.6	0.6–0.7	0.6	
C18:0	9.6	8.5–11.0	12.2	10.4–14.6
C20:0	0.5	0.4–0.5	0.4	
C10:1	0.3	0.2–0.3	0.2	
C14:1	0.3	0.2–0.5	0.8	0.5–1.1
C16:1	1.0	0.7–1.3	1.5	1.4–2.0
C17:1	0.2	0.2–0.3	0.4	
cis C18:1	18.2	15.3–19.8	17.2	14.9–22.0
trans C18:1	2.9	2.5–3.2	3.9	
C18:2	2.3	1.9–2.5	1.4	1.2–1.7
C18:2 conjugated	0.7	0.6–1.0	1.1	0.8–1.5
C18:3	0.8	0.6–1.0	1.1	0.8–1.5

Source: adapted from Recio *et al*. (2009), with permission of Blackwell Publishing, and based on data from Walstra & Jennes (1984) and Ramos & Juárez (2011).

which liberate a constant and elevated supply of butyrate to the colon, are most effective in preventing chemically induced colon tumours. Moreover, the level of butyric acid in the colonic lumen of patients with colorectal cancer and adenomas was found to be lower than that in healthy individuals (Parodi, 2004). Synergism between butyrate and other dietary components and common drugs in reducing cancer cell growth has also been shown. A summary of these related cell-growth inhibiting effects induced by butyric acid is outlined by Parodi (2006).

With regard to C6:0, C8:0 and C10:0, Marten *et al*. (2006) reported their potential to reduce body weight and body fat. These fatty acids are particularly digestible, as they are hydrolysed preferentially from TAGs and are transferred directly from the intestine to the portal circulation without resynthesis of TAGs. Thus, there is only a low tendency for adipose formation. Furthermore, these compounds are a preferred source of energy (β-oxidation). Given in moderate amounts, in diets with limited fat supply, they may actually reduce fasting lipid levels more than oils rich in mono-unsaturated or polyunsaturated fatty acids (Marten *et al*., 2006). On the other hand, C4:0, C6:0, C8:0 and C10:0 fatty acids also exert antimicrobial and antiviral activities in both *in vitro* and *in vivo* animal studies (Neyts *et al*., 2000).

Stearic acid (C18:0), with an average concentration in sheep milk fat of 10% (Table 25.3), is considered neutral from the point of view of human health and can reduce plasma cholesterol as well as oleic acid (*cis*-9 C18:1).

25.2.2.2 Unsaturated fatty acids

Oleic acid, the second most predominant fatty acid in sheep milk fat (Table 25.3), is regarded as an antiatherogenic

agent. Human diets high in oleic acid are mostly reported to decrease the level of LDL-cholesterol, whereas levels of high-density lipoprotein (HDL)-cholesterol are not affected significantly (Molkentin, 2000). The PUFAs in sheep milk fat mainly comprise linoleic acid (*cis*-9, *cis*-12 C18:2) and α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3), as well as smaller amounts of their positional and geometric isomers. Both are essential fatty acids, have many diverse functions in human metabolism and overall promote an antiatherogenic effect. Mean linoleic acid content accounts for 70–75% of the total C18:2, excluding conjugated C18:2, in sheep milk, whereas the rest of the *trans* C18:2 isomer group represents slightly more than one-quarter of this fraction and 0.5–0.9% of the total fatty acids (Goudjil *et al*., 2004). On average, α-linolenic acid rarely exceeds 1% (Table 25.3). However, other omega-3 PUFAs are hardly found in sheep milk fat, except when animal diets are supplemented with marine oil sources (Reynolds *et al*., 2006; Toral *et al*., 2010a, b).

25.2.2.3 Trans fatty acids

The contents of *trans* fatty acids (TFAs) in sheep milk fat range from 2.5 to 5% of total fatty acids, mainly depending on diet and season. Monoene TFAs are the most abundant in all species and the pattern of *trans* C18:1 isomer distribution is similar (Precht *et al*., 2001). TFAs in dairy fat are not seen as bioactive lipids in a positive sense; however, since TFAs have come under scrutiny due to their influence on lipid levels and on other risk factors for coronary heart disease (CHD) and CVD, the question of whether all TFAs are alike or whether the most abundant TFA isomers from dairy fat have metabolic properties distinct from those of other origins (hydrogenation reactions for instance) has gained increasing relevance (Jakobsen *et al*., 2008; Shingfield *et al*., 2008; Gebauer *et al*., 2011).

The main source of TFAs consumed daily by humans is partially hydrogenated vegetable fats and oils (Gebauer *et al*., 2011), although these compounds also occur naturally in ruminant milk as a result of partial biohydrogenation of PUFAs caused by rumen microorganisms. There is a considerable overlap of TFA isomers in fats of ruminant origin and partially hydrogenated vegetable oils, with many isomers in common. However, the isomer profile of hydrogenated vegetable fats is very different. During the hydrogenation of vegetable fats a wide range of *trans* monounsaturated fatty acids are principally formed (e.g. *trans*-9 C18:1, elaidic acid and *trans*-10 C18:1) while the main TFA in milk fat is *trans*-11 C18:1, vaccenic acid (VA) (International Dairy Federation, 2005). The importance of VA lies in its role as a precursor of the main isomer of CLA, rumenic acid (RA, *cis*-9, *trans*-11 C18:2), physiologically the most relevant bioactive compound present in milk fat. This synthesis occurs not only in the ruminant mammary gland (Griinari & Bauman, 1999; Bichi *et al*., 2012) but also in human tissues (Turpeinen *et al*., 2002; Kuhnt *et al*., 2006; Mosley *et al*., 2006). The proportion of VA in milk fat total monoene TFAs in sheep milk is around 45–60% (Precht *et al*., 2001; Goudjil *et al*., 2004; Gómez-Cortés *et al*., 2009) whereas elaidic acid and *trans*-10 C18:1 are present in considerably smaller amounts (average 5% and 10%, respectively, of total monounsaturated TFAs). Thus, in contrast to the majority of hydrogenated vegetable oils enriched in *trans*-10 and *trans*-9 C18:1, the consumption of dairy products represents a very low intake of these components.

Individual TFA isomers could have differing physiological effects. There is evidence of unfavourable effects of TFAs from hydrogenated vegetable oils on LDL and other risk factors of atherosclerosis, whereas the predominant TFA in milk – VA – would not exert these detrimental effects (International Dairy Federation, 2005; Field *et al*., 2009; Malpuech-Brugère *et al*., 2010). Most of the studies have reported that the positive association with risk of CHD could be explained entirely by the intake of TFAs from hydrogenated vegetable oils. Several of the large prospective studies, which established the notion that intake of TFAs increases CHD risk, showed a significant inverse association with intake of animal or dairy TFAs, a non-significant inverse trend or at least no change with increasing intake of TFAs from such sources (Pfeuffer & Schrezenmeir, 2006; Gebauer *et al*., 2011). In addition, Tricon *et al*. (2006) and Wanders *et al*. (2010) found that increments in the concentrations of VA and RA are not related to CVD.

Results from an acute oral safety study in rats (Anadón *et al*., 2010) showed that a dairy fat rich in VA (14%) obtained from sheep significantly decreased TAGs in plasma and did not result in any detrimental metabolic effects or negatively influence any toxicological parameters. On the contrary, administration to the rats of a sheep milk fat with high content of *trans*-10 C18:1 (20%) promoted triglyceridaemia. More research has encouraged awareness of the healthy effects of consuming VA, as emerging data suggest that consumption of this TFA may impart benefits beyond those associated with CLA (Field *et al*., 2009).

25.2.2.4 Conjugated linoleic acid

The generic name ‘conjugated linoleic acid’ is a collective term embracing all positional and geometric isomers of linoleic acid that contain a conjugated double bond system. Data from *in vitro* studies and animal models have been used to suggest that the RA isomer is responsible for CLA’s anticarcinogenic and antiatherogenic properties, as well as a multiplicity of potentially beneficial effects on human

Table 25.4. Conjugated linoleic acid (CLA) isomers (% total CLA) in sheep milk and Manchego cheeses manufactured from pure sheep milk.

Isomer	Milk (Luna *et al*., 2005a)	Manchego cheeses (Luna *et al*., 2008)
trans-12, *trans*-14	1.31–3.47	1.59–2.06
trans-11, *trans*-13	1.21–5.08	2.13–2.66
trans-10, *trans*-12	1.17–1.77	1.10–1.35
trans-9, *trans*-11	1.13–1.99	1.30–1.52
trans-8, *trans*-10	1.05–1.37	0.53–0.68
trans-7, *trans*-9	0.48–0.61	0.29–0.38
12–14 (*cis–trans* plus *trans–cis*)	0.52–1.83	0.79–1.15
11–13 (*cis–trans* plus *trans–cis*)	0.76–4.23	1.31–2.13
10–12(*cis–trans* plus *trans–cis*)	0.28–0.41	0.37–0.56
9–11 (*cis–trans* plus *trans–cis*)	76.5–82.4	79.50–81.7
8–10 (*cis–trans* plus *trans–cis*)	0.11–0.71	—
7–9 (*cis–trans* plus *trans–cis*)	3.31–9.69	6.94–7.11

Source: based on data from Luna *et al*. (2005a, 2008).

health (Lee *et al*., 2005; Bhattacharya *et al*., 2006; Yurawecz *et al*., 2006). From initial studies showing the anticarcinogenic effects of CLA (inhibition of epithelial tumours in animals), a large number of research studies have been performed to determine its biological and physiological properties (Parodi, 2008). There are many possible metabolic pathways involved in the biological activity of CLA. It has been suggested that CLA competes with arachidonic acid (C20:4) in the cyclooxygenase reaction, thus reducing the concentration of prostaglandins and thromboxanes of the 2-series (Akahoshi *et al*., 2004). CLA may suppress cyclooxygenase gene expression and reduce the release of proinflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin in animals, and may also activate peroxisome proliferator-activated receptor (PPAR) transcription factors and reduce the initial step in the activation of NF-κB thereby reducing cytokines, adhesion molecules and others induced by stress (Cheng *et al*., 2004).

Among ruminants, sheep milk fat contains not only one of the highest levels of RA, but also the major content of VA, its physiological precursor. In first studies (Jahreis *et al*., 1999) mean total CLA content decreases in the following order: sheep>cow>goat milk fat (1.2, 0.7 and 0.6% of total fatty acids, respectively). Other reports on sheep milk fat (Prandini *et al*., 2001; Barbosa *et al*., 2003) have quantified the most prominent component assigned to CLA by gas chromatography (GC). However, this main GC peak includes more than one component and minor CLA isomers masked by the RA peak. The combination of GC/MS of fatty acid methyl esters and 4,4-dimethyloxazoline derivatives and silver-ion high-performance liquid chromatography (Ag^+-HPLC) of fatty acid methyl esters helped to reveal the CLA isomer profile in sheep milk (Luna *et al*., 2005a). Table 25.4 shows the range of the relative composition of CLA isomers in sheep dairy fat determined by Ag^+-HPLC. RA represents more than 75% of total CLA, whereas *trans*-7, *cis*-9 C18:2 is, from a quantitative viewpoint, the second most abundant CLA molecule (5–10% of total CLA). Minor amounts of other CLA isomers with different positional and geometric configurations can also be found.

Besides RA, other CLA isomers have been associated with several metabolic processes related to health. The isomer *trans*-10, *cis*-12 C18:2 has lean-body-mass-enhancing properties (Belury, 2002; Pariza, 2004) and several studies in animals and humans have suggested that this isomer could be responsible for decreasing glucose levels and increased insulin resistance (Khanal, 2004). However, the amount of this isomer in sheep milk fat is very low, less than 1% of total CLA (Table 25.4).

Information on the biological activity of other CLA minority isomers detected in sheep milk fat is very scant. The isomer *cis*-9, *cis*-11 C18:2 has been shown to block estrogen signalling in human breast cancer cells using *in vitro* assays (Tanmahasamut *et al*., 2004). Other studies have reported the potent inhibitory effect of *trans*-9, *trans*-11 C18:2 on the growth of human colon cancer cells (Beppo *et al*., 2006) as well as antiproliferative and pro-apoptotic effects on bovine endothelial cells (Lai *et al*., 2005). However, in this field, further research is needed.

There is great interest in increasing CLA content and changing the fatty acid profile in dairy products to provide value-added foods. Processing of sheep milk to cheese

appears to have no effect on the final concentration of CLA, and the isomeric profile and content of CLA is primarily dependent on the CLA level in the unprocessed milk (Luna *et al*., 2005b, 2007, 2008). On the other hand, no other factors (e.g. breed, parity, lactation length) can substantially affect the CLA content in milk fat (Tsiplakou *et al*., 2006), which indicates that dietary factors remain sovereign in explaining the high CLA content variability in sheep milk fat.

The most important of the intrinsic and extrinsic variables that modulate the fatty acid composition of sheep milk is the feed, and in particular supplementation of the diet with lipid (Bocquier & Caja, 2001; Pulina *et al*., 2006; Sanz-Sampelayo *et al*., 2007). Changes in the fatty acid profile of ovine milk fat should not substantially differ from the pattern previously described for cow milk. Milk CLA concentration of different ruminant species varies with the season, mainly due to variations in feeding factors. The greatest seasonal differences in CLA concentration in sheep milk range from 1.28% in summer to 0.54% at the end of the winter period (Jahreis *et al*., 1999). The effect of feeding fresh forages or Mediterranean pastures and of season (related to changes in pasture quality) on the fatty acid composition of sheep milk, with special emphasis on the content of CLA and its precursors, has been reported by Addis *et al*. (2005), Cabiddu *et al*. (2005) and Nudda *et al*. (2005).

In addition to enhancing CLA content, the addition of vegetable seeds and oils to the diet also results in milk fat containing a lower proportion of SFAs and greater amounts of monounsaturated fatty acids (including VA) and PUFAs (Antongiovanni *et al*., 2004; Luna *et al*., 2005b; Zhang *et al*., 2006). More recent studies have confirmed the feasibility of a complete systems approach for the production of sheep milk and cheese enriched in CLA and omega-3 fatty acids (Gómez-Cortés *et al*., 2009; Mele *et al*., 2011). The RA and VA contents of milk and cheese from sheep receiving a ration rich in extruded linseed were threefold higher than in milk from ewes fed a control ration. Additionally, supplementation of sheep feed with linseed increased the α-linolenic acid content in milk fivefold and reduced the concentration of SFAs (C12:0, C14:0 and C16:0) by 15–30%; other SFAs (C4:0 to C10:0) increased significantly. Furthermore, consumer acceptability attributes of CLA-enriched cheese manufactured from milk of sheep fed a lipid-supplemented diet were not different from those of cheese manufactured from milk of animals fed non-supplemented diets (Gómez-Cortés *et al*., 2009).

It has also been reported in cows that marine oil is more effective than plant lipids for enhancing milk fat CLA content, and these responses can be further increased when fish oil is fed in combination with supplements rich in linoleic acid (Stanton *et al*., 2003). However, to date, data on CLA enhancement in sheep milk by the addition of fish oil supplements are very limited (Reynolds *et al*., 2006; Toral *et al*., 2010a,b).

25.2.3 Other minor lipid compounds

Along with TAGs, sheep milk contains complex lipids (phospholipids) and different liposoluble compounds (sterols, β-carotene, vitamins) with biological activity. Phospholipids are associated with the MFGM and account for 0.2–1% of total milk lipids (Rombaut & Dewettinck, 2006; Rodríguez-Alcalá & Fontecha, 2010). Sphingomyelin and its metabolites, ceramide and sphingosine, are reported to have tumour-supressing properties by influencing cell proliferation and are highly bioactive compounds with bacteriostatic and cholesterol-lowering properties (Parodi, 2004, 2006; Gustavsson *et al*., 2010). Further, some phospholipids exhibit antioxidative properties in dairy fat products with low water content (Molkentin, 2000). However, to date, only very limited data are available on the phospholipid content in dairy products and the influence of processing and environmental variables on their concentration and relative distribution. The proportions of corresponding phospholipid classes in sheep milk are remarkably similar to milk of other ruminants (MacGibbon & Taylor, 2006). Phosphatidylethanolamine, phosphatidylcholine and sphingomyelin are the most abundant, with smaller amounts of phosphatidylinositol and phosphatidylserine.

Sterols are a minor fraction of sheep milk total lipids, the main sterol being cholesterol (about 300 mg/100 g fat, equivalent to approximately 10 mg/dL milk). Values reported for the cholesterol content of sheep milk vary considerably, associated with the breed and partly due to the use of different analytical techniques. Small amounts of other sterols implicated in cholesterol biosynthesis have also been found in ovine milk: lanosterol (5–15 mg/100 g fat) and, in even smaller proportions, dihydrolanosterol, desmosterol and lathosterol (Goudjil *et al*., 2003b).

The sterol fraction of milk is of nutritional interest because high levels of cholesterol in human blood plasma are associated with an increasing risk of CVD. Nevertheless, it is known through analysis of the available epidemiological and clinical data that for the general population, dietary cholesterol makes no significant contribution to atherosclerosis and risk of CVD (McNamara, 2000). Cholesterol is also important for the resorption of fats and its role as precursor in the synthesis of steroid hormones.

Other molecules present in the milk lipid fraction at low amounts have been claimed as bioactive components. Sheep milk fat contains a small amount of ether lipids (alkyldiacylglycerols and alkylacylphospholipids)

(Hallgren *et al*., 1974). These compounds and their derivatives have potent antitumour activity. It is believed that ether lipids are incorporated and accumulated in cell membranes and thereby influence biochemical and biophysical processes (Parodi, 2006). There is also substantial epidemiological evidence for an association between diets rich in vitamin A and β-carotene and a decreased risk of cancer (Parodi, 2006). Ovine milk contains virtually no β-carotene but supplies adequate amounts of vitamin A; indeed, vitamin A levels are higher than those found in cow milk (Park *et al*., 2007). Notwithstanding, little information about these topics is found in the literature. Thus, more research should be undertaken in this field, as this would lead to a better knowledge of the raw materials available and a deeper insight into the contribution of sheep dairy products in maintaining human health.

25.3 PROTEINS AND THEIR BIOLOGICAL FUNCTIONS

The protein components of milk have multiple functions. Milk provides all essential amino acids, which are necessary for growth and development. In addition, milk proteins exhibit a range of biological activities that influence digestion, metabolic responses to absorbed nutrients, growth and development of specific organs, and resistance to disease. Hydrolysis of these milk proteins, for instance during food digestion, releases a large number of different protein fragments capable of exerting specific biological activities, such as antihypertensive, antimicrobial, opioid, antioxidant, immunomodulatory or mineral-binding effects (Madureira *et al*., 2010; Hernández-Ledesma *et al*., 2011a). Many different active peptides have been found within the sequences of bovine milk proteins. Since the primary structures of ovine milk proteins show great homology with those observed for bovine milk proteins, it can be predicted that ovine proteins should also be a good source of bioactive peptides. The research advances pertaining to the biological properties of ovine and caprine whey proteins and derived peptides have recently been reviewed by Hernández-Ledesma *et al*. (2011b).

Caseins constitute the major protein fraction in ovine milk (76–83% of total proteins) and are present in most types of cheese. The main physiological role of caseins is to deliver amino acids for nutrition and to transport a large number of the minerals the newborn requires for bone growth (e.g. Ca, Mg, phosphate, Fe, Zn, Cu). Caseins comprise four types: α_{s1}, α_{s2}, β and κ. The heterogeneity of caseins is determined either by the presence of genetic variants or by other factors such as discrete phosphorylation level, variation in the extent of glycosylation of the κ-casein fraction, and the coexistence of proteins with different chain lengths (Ramos & Juárez, 2011).

A review about the genetic polymorphism of ovine milk proteins and its influence on the technological properties of milk has been published by Amigo *et al*. (2000). It has been demonstrated that the four ovine casein genes (α_{s1}, α_{s2}, β and κ) are polymorphic and linked in both the ovine and bovine genome. The α_{s1}-casein is a mixture of two molecular species (199 and 191 residues, respectively) that differ in the deletion of residues 141–148. Five variants of α_{s1}-casein have been described. These are designated A, B, C, D and E, in line with the nomenclature proposed for cow and goat milk caseins (Chianese *et al*., 1996). The α_{s1}-casein variant D is the only variant that was described in the last century; it was called the Welsh variant in light of its discovery in the Welsh mountains by King in 1966. This is the least phosphorylated variant. The α_{s1}-casein variant C differs from variant A in the substitution of Ser for Pro at position 13, which determines loss of the phosphate group on site 12 of the protein chain. Recently, three new variants F, G and H have been described in German sheep breeds (Glambra *et al*., 2010).

The α_{s2}-casein has 208 amino acid residues compared with 207 for the cow counterpart. Two variants of α_{s2}-casein, A and B, have been described, differing in that Asn49 and Lys200 are replaced by Asp49 and Asn200. In addition, a variant presenting high electrophoretic mobility and low molecular weight has been found in the Manchega breed (Chianese *et al*., 1993). Recently, a new variant has been described in which Asn200 has been replaced by Lys200 (Chessa *et al*., 2010).

Ovine β-casein comprises 209 amino acids. For β-casein there is a non-genetic polymorphism due to varying degrees of phosphorylation, with six and five phosphate groups for β1 and β2, respectively. In addition, three genetic variants, designated A, B and C, have been described (Chianese, 1997). The only sequence difference found between variants A and C was substitution of the amino acid Glu at position 2 in variant A for Gln in variant C, but no sequence data for the B variant are yet available. Both the stability of the micelle and the availability and distribution of calcium will be affected by the extent of phosphorylation of the caseins. Chessa *et al*. (2010) found two additional variants using polymerase chain reaction single-stranded conformation polymorphism.

Sequencing of ovine κ-casein has shown that it comprises 171 amino acid residues. No genetic variants have been found in κ-casein, whereas it presents non-genetic polymorphism due to varying degrees of glycosylation at three different Thr residues (positions 135, 137 and 138) and two phosphorylation sites (Ser151 and Ser168). The casein fraction also contains γ-caseins, the product of the breakdown of β-caseins by plasmin.

The major whey proteins are β-lactoglobulin (BLG), α-lactalbumin (ALA) and seroalbumin. Other minor proteins, such as immunoglobulins, transferrin, lactoferrin, proteose peptone, lactoperoxidase, lysozyme, calmodulin (calcium-binding protein), prolactin and folate-binding protein, have been described (Park *et al*., 2007). In the case of rennet whey, caseinomacropeptide (CMP) is also present.

BLG is a small, soluble and globular protein containing 162 amino acids in a single peptide chain with a molecular mass of 18.3 kDa. It is the major whey protein in ruminant milk and is involved in the transport of retinol in blood serum. In addition, it has been shown that BLG enhances intestinal uptake of retinol, TAGs, and long-chain fatty acids in pre-ruminant calves (Kushibiki *et al*., 2001), and it has been speculated that this protein may play a role in the absorption and subsequent metabolism of fatty acids. Other possible functions have been described for this whey protein, such as its role in developing passive immunity with IgG (Sutton & Alston-Mills, 2006). Wong *et al*. (1998) demonstrated that this whey protein stimulates proliferation of normal murine spleen cells and production of immunoglobulins. Moreover, BLG is a rich source of cysteine, an essential amino acid that appears to stimulate glutathione synthesis, an anticarcinogenic tripeptide produced by the liver for protection against intestinal tumours (Mcintosh *et al*., 1995).

ALA is a small globular protein of approximately 14 kDa that consists of a single polypeptide chain with eight cysteine residues. It is physiologically important because of its role in lactose synthesis. In the last decades, it has been demonstrated that whey proteins are superior to other dietary proteins for suppression of tumour development due to the components lactoferrin, BLG, ALA and seroalbumin (Parodi, 2007). The best known is the antitumoral activity observed for the complex that contains human ALA and oleic acid called HAMLET (human α-lactalbumin made lethal to tumour cells). This complex is able to kill tumour cells by a process resembling programmed cell death. HAMLET has broad antitumour activity *in vitro*, and its therapeutic effect has been confirmed *in vivo* in a human glioblastoma rat xenograft model, in patients with skin papillomas and in patients with bladder cancer (Hallgren *et al*., 2008). Likewise, a complex of bovine ALA and oleic acid (BAMLET) killed tumour cells via a mechanism involving lysosomal membrane permeabilisation, showing potent cytotoxic activity against eight different cancer cell lines (Rammer *et al*., 2010). In recent years, a variety of HAMLET-like substances, termed XAMLET by Zhang *et al*. (2010) including a complex comprising ALA (bovine, equine, porcine, ovine and caprine) and oleic acid, have been demonstrated to exert similar biological activities to HAMLET (Spolaore *et al*., 2010).

Immunoglobulins were among the first host protein defence systems described. Immunoglobulins constitute a complex group of globular proteins produced by B lymphocytes that protect the gut mucosa against pathogenic microorganisms, also providing protection against diseases in the ruminant neonate until its own immune system is developed. Recently, it has been demonstrated that oral administration of ovine serum immunoglobulin modulates aspects of immunity such as phagocytosis, lymphocyte proliferation, cytokine production, and intestinal and plasma immunoglobulin concentrations in growing rats (Balan *et al*., 2010). These authors had previously reported the effect of this ovine immunoglobulin fraction in improving growth performance, organ weight and gut morphology in growing rats (Balan *et al*., 2009).

Lactoferrin is a globular multifunctional protein that binds, transports and supplies iron in the organism. The iron-binding properties seem to vary between lactoferrin from different species. The levels of this protein in sheep milk are slightly higher than in cow milk, with values of approximately 0.1 mg/mL. Lactoferrin exhibits activity as an antimicrobial agent for host defence and as a physiological regulator with respect to both inflammatory and immune responses. Several reviews have been published where the various physiological functions of the protein are addressed, as well as the important *in vivo* experimental results that promote its use in upregulating mucosal immune responses (Wakabayashi *et al*., 2006; Kanwar *et al*., 2009). In summary, lactoferrin is the most important and widely studied milk protein for human health. It has antibacterial, antifungal, antiviral, antiparasite and antitumour activities and accelerates immunomodulatory properties. In addition, lactoferrin is a potent inhibitor for several enveloped and naked viruses, such as rotavirus, enterovirus and adenovirus. Recent research has revealed that bovine lactoferrin induces apoptosis of human stomach cancer cells (Xu *et al*., 2010).

Lysozyme and lactoperoxidase are also important antimicrobial proteins found in mammalian milk and colostrums. Lysozyme is mainly active against Gram-positive microorganisms whereas Gram-negative microorganims containing catalase, such as *Pseudomonas*, coliforms, salmonellae and shigellae, are killed by activated lactoperoxidase provided that the hydrogen peroxide substrate is present in excess. Recently, lactoperoxidase has been identified in the milk basic protein fraction as an inhibitor of osteoclastogenesis (Morita *et al*., 2011). This fraction has also been shown to have a direct effect on strengthening of bones in healthy human volunteers (Uenishi *et al*., 2007).

In recent years, studies have focused on proteins of the MFGM and their functions. One of the proteins isolated from MFGM, termed fatty acid binding protein, has been shown to inhibit some breast cancer lines (Spitsberg, 2005). In addition, Pisanu *et al*. (2011) applied the proteomic approach to sheep MFGM proteins and identified 140 proteins. Comparison of these data with the cow MFGM proteome showed a higher presence of cytoplasmic and secreted proteins in sheep than in bovine milk.

25.3.1 Bioactive peptides derived from sheep milk proteins

Enzymatic hydrolysis of milk proteins can release fragments able to exert specific biological activities, such as antihypertensive, antimicrobial, opioid, antioxidant, immunomodulatory or mineral-binding effects. Such protein fragments, known as bioactive peptides, are released from the precursor protein during gastrointestinal digestion and/or during food processing (FitzGerald & Murray, 2006). Because of their physiological and physicochemical versatility, milk peptides are regarded as highly important components for health-promoting foods or pharmaceutical applications. The potential of these bioactive peptides to reduce the risk of chronic diseases and to promote human health has aroused increasing scientific and commercial interest over the past decade. Research in the field of bioactive peptides has mainly been focused on milk proteins of bovine origin and has been extensively reviewed (López-Fandiño *et al*., 2006; Korhonen, 2009; Hernández-Ledesma *et al*., 2011a; Mills *et al*., 2011). However, during recent years, research has been extended to milk proteins from other mammals, including ovine and caprine species (Park *et al*., 2007; Recio *et al*., 2009; Hernández-Ledesma *et al*., 2011b). An update of bioactive peptides obtained from ovine milk is provided in the following sections.

25.3.1.1 Antihypertensive peptides

Among the bioactive peptides known, those with angiotensin-converting enzyme (ACE)-inhibitory properties are receiving special attention due to their potential beneficial effects in the treatment of hypertension. ACE is a multifunctional enzyme, located in different tissues, and is able to regulate several systems that affect blood pressure. It is responsible for generating vasopressor angiotensin II and for the inactivation of the vasodepressor bradykinin. Much work has been done to evaluate the *in vitro* activity of peptides on ACE activity. However, peptides with ACE-inhibitory activity *in vitro* do not necessarily exert antihypertensive activity after oral ingestion. Discrepancies between ACE-inhibitory activity and antihypertensive peptides can be due to further degradation during gastrointestinal digestion, failure to reach the target organ in sufficient amounts, or because mechanisms other than ACE inhibition may be involved. Therefore, it is important to distinguish both *in vitro* and *in vivo* effects. The antihypertensive effects can be assessed by *in vivo* experiments using the spontaneously hypertensive rat (SHR) and human studies to determine whether these peptides posses an antihypertensive effect in human subjects. Table 25.5 shows ACE-inhibitory and antihypertensive activity in SHRs of peptides from caseins and whey proteins from ovine milk and obtained by fermentation or enzymatic hydrolysis.

Milk fermentation has already been proven to be a successful strategy for producing ACE-inhibitory and antihypertensive peptides. Because sheep milk is mainly used for cheesemaking, the formation of bioactive peptides during cheese ripening is of special interest. Several ACE-inhibitory peptides have been isolated from extracts of Italian cheeses (Gobbetti *et al*., 2004) and from a Spanish Manchego cheese prepared by inoculating ovine milk with *Lactococcus lactis* subsp. *lactis* and *Leuconostoc mesenteroides* (Gómez-Ruiz *et al*., 2002). In this work, 22 peptides from α_{s1}-, α_{s2}- and β-casein were sequenced by tandem mass spectrometry comprising several active chromatographic fractions. One of the peptides identified is α_{s1}-casein f(102–109) KKYNVPQL. This peptide exhibited considerable ACE-inhibitory activity with IC_{50} values of 77.1 μmol/L (Gómez-Ruiz *et al*., 2004). Recently, Miguel *et al*. (2010) evaluated the changes in arterial blood pressure after single oral administration of the α_{s1}-casein KKYNVPQL peptide in SHRs. The peptide did not modify systolic blood pressure in SHRs and caused a slight but significantly maintained decrease in diastolic blood pressure that returned to baseline values 24 hours after administration of peptides. This peptide is hydrolysed by gastrointestinal proteases in several fragments, causing a significant decrease in the *in vitro* ACE-inhibitory activity of the peptide (Gómez-Ruiz *et al*., 2004). The cleavage of these sequences could occur in the gastrointestinal tract and therefore other shorter fragments derived from KKYNVPQL are probably responsible for the antihypertensive effect observed when this peptide was orally administered.

The best-characterised ACE-inhibitory peptides are VPP and IPP found in milk fermented with *Lactobacillus helveticus* and commercialised in Japan (Ameal S®/Calpis®, Calpis Co. Ltd, Tokyo, Japan) and Finland (Valio Evolus® Double Effect, Valio Ltd, Finland). This fermented milk has shown beneficial effects on blood pressure in several rat models and human studies. Recently, Cicero *et al*. (2011) in a meta-analysis of 18 randomised controlled trials found significant decreases in systolic and diastolic blood pressure (–3.73 mmHg and –1.97 mmHg, respectively). VPP and IPP have also been identified and

Table 25.5. ACE-inhibitory and antihypertensive activity determined in spontaneously hypertensive rats of peptides derived from ovine caseins and whey proteins.

Peptide fragment	Sequence*	IC_{50} (μmol/L)†	Maximum decrease in SBP (mmHg)	Origin	Reference
α_{s1}-Casein f(102–109)	KKYNVPLQ	77.1	–11.5	Manchego cheese	Gómez-Ruiz *et al.* (2007), Miguel *et al.* (2010)
α_{s2}-Casein f(203–208)	PYVRYL	1.9	–23.4	Hydrolysis of ovine casein with pepsin	Recio *et al.* (2005)
β-Casein f(58–68)	LVYPFTGPIPN	10	–28.0	Kefir from caprine milk	Quirós *et al.* (2005), Miguel *et al.* (2010)
β-Casein f(74–76)	IPP	5.0	–28.3	Milk fermentation with *Lactobacillus helveticus*, *Saccharomyces cerevisiae*	Nakamura *et al.* (1995)
β-Casein f(84–86)	VPP	9.0	–32.1	Milk fermentation with *L. helveticus*, *S. cerevisiae*	Nakamura *et al.* (1995)
κ-Casein f(22–24)	IAK	15.7	–20.7	Hydrolysis with digestive enzymes	Miguel *et al.* (2010)
κ-Casein f(61–66)	YAKPVA	14.3	–23.1	Hydrolysis with digestive enzymes	Miguel *et al.* (2010)
κ-Casein f(76–86)	WQVLPNAVPAK	10.1	–18.4	Hydrolysis with digestive enzymes	Miguel *et al.* (2010)
κ-Casein f(76–86)	HPHPHLSF	28.9	–18.4	Hydrolysis with digestive enzymes	Miguel *et al.* (2010)
κ-Casein f(106–112)	MAIPPKK	4785	–28.0	Hydrolysis with trypsin (caseinomacropeptide)	Miguel *et al.* (2007)
ALA f(50–53)	YGLF	733	–23.0	Hydrolysis with gastric and pancreatic enzymes	Nurminen *et al.* (2000)
BLG f(58–61)	LQKW	34.7	–18.1	Hydrolysis with thermolysin	Hernández-Ledesma *et al.* (2007)
BLG f(78–80)	IPA	141	–31.0	Hydrolysis with proteinase K	Abubakar *et al.* (1998)
BLG f(103–105)	LLF	79.8	–20.0	Hydrolysis with thermolysin	Hernández-Ledesma *et al.* (2007)
BLG f(142–145)	ALPM	928	–21.4	Commercial whey product	Murakami *et al.* (2004)

*One letter amino acid sequence.

†Peptide concentration needed to inhibit 50% of the original angiotensin-converting enzyme activity.

ALA, α-lactalbumin; BLG, β-lactoglobulin; SBP, systolic blood pressure (mean value).

Source: based on data from Hernández-Ledesma *et al.* (2011a).

quantified in different cheese varieties by Bütikofer *et al*. (2007) who found, in some cheese varieties, physiologically relevant amounts. However, a large variation exists between samples of the same cheese variety depending on the ripening time or the cheesemaking process, as well as between different varieties. In Roquefort and Manchego cheese made from ovine milk, the quantity of VPP and IPP was moderate (below 50 mg/kg) (Bütikofer *et al*., 2007).

The production of antihypertensive peptides has also been accomplished by enzymatic hydrolysis of milk proteins. Combined action of pepsin, chymotrypsin and trypsin was required to liberate peptide fragments from κ-casein with potent ACE-inhibitory and antihypertensive activity in SHRs (Miguel *et al*., 2010). Peptides IAK, YAKPVA and WQVLPNAVPAK showed a clear decrease in both systolic and diastolic blood pressure (Table 25.5). However, peptide HPHPHLSF caused a significant decrease in diastolic blood pressure in the SHR, but this sequence did not modify the systolic blood pressure of these animals. Of special interest is the ovine α_{s2}-casein fragment PYVRYL, produced by hydrolysis of α_{s2}-casein with pepsin, that exhibited potent *in vitro* ACE-inhibitory activity (IC_{50} 2.4 μmol/L) (López-Expósito *et al*., 2007) and significant antihypertensive activity in SHRs (Recio *et al*., 2005).

With regard to whey proteins, hydrolysis with a combination of digestive enzymes or with highly proteolytic and less specific enzymes, such as thermolysin, produce peptides with ACE-inhibitory activity. Two potent ACE-inhibitory peptides, LLF and LQKW, were identified in a caprine BLG hydrolysate with thermolysin (Hernández-Ledesma *et al*., 2002). Subsequently, the antihypertensive effect of these two peptides in SHRs has been reported (Hernández-Ledesma *et al*., 2007) and it is important to highlight that these domains are maintained in ovine BLG. Chobert *et al*. (2005) investigated the ACE-inhibitory activity of ovine BLG hydrolysed with trypsin, and of yoghurts made from ovine milk using different starters. A higher susceptibility for BLG variant B to tryptic hydrolysis was found than for variant A, as previously observed for pepsin (El-Zahar *et al*., 2005). In addition, several peptides from this tryptic hydrolysate were identified by tandem mass spectrometry. Interestingly, the ACE-inhibitory activity after fermentation of ovine milk was higher than that obtained after tryptic hydrolysis of ovine BLG, showing that hydrolysis of caseins with enzymes of bacterial origin can constitute an efficient substrate and procedure for the formation of ACE-inhibitory peptides.

Several ACE-inhibitory peptides from ALA have been identified. Mullally *et al*. (1996) synthesised peptides from the ALA and BLG, and studied their ACE-inhibitory activity. Despite none of these peptides showing strong inhibitory activity, the cardiovascular effects of the tetrapeptide YGLF, known as α-lactorphin, was later demonstraed in SHRs. α-Lactorphin, a peptide also produced by enzymatic hydrolysis with pepsin and trypsin, dose dependently lowered blood pressure without affecting heart rate in SHRs and provided evidence for the involvement of opioid receptors in its depressor action (Nurminen *et al*., 2000). Further studies to shed light on the antihypertensive mechanism of this tetrapeptide showed that its beneficial effect was directed towards endothelial function, improving vascular relaxation in adult SHRs *in vitro* (Sipola *et al*., 2002). This peptide, α-lactorphin, has also been obtained after protein hydrolysis of caprine ALA (Bordenave *et al*., 2000).

ACE-inhibitory peptides have also been identified in hydrolysates derived from ovine and caprine CMP. Manso and López-Fandiño (2003) found that undigested bovine, caprine and ovine CMP exhibited moderate ACE-inhibitory activity, but this increased considerably after digestion under simulated gastrointestinal conditions. The ACE-inhibitory peptides MAIPPK and MAIPPKK, corresponding to κ-casein f(106–111) and f(106–112) respectively, were identified via proteolysis with trypsin. The peptide MAIPPKK at a dose of 10 mg/kg significantly lowered blood pressure in SHRs, with a maximum decrease after 8 hours (28 mmHg). It can be hypothesised that IPP could be responsible for the antihypertensive effect of MAIPPKK. This peptide also evoked significant relaxation in endothelial-intact aortic ring preparations (Miguel *et al*., 2007). These findings might help to promote further exploitation of CMP as multifunctional active ingredient, broadening the potential uses of rennet whey from various sources.

The presence of ACE-inhibitory peptides has also been investigated in other food matrices, such as ovine yoghurt and hydrolysates of ovine milk proteins. Several previously described active sequences have been found in sheep milk yoghurt produced with a yoghurt culture enriched with a probiotic strain (Papadimitriou *et al*., 2007). Indeed, a peptide derived from β-casein, YPVEPFTE, with well-established ACE-inhibitory and opiate-like activity, was identified in this probiotic yoghurt.

25.3.1.2 Antimicrobial peptides

Bioactive proteins and peptides derived from milk have been reported to provide non-immune disease defence and control of microbial infections (McCann *et al*., 2006). It is generally accepted that the total antibacterial effect in milk is greater than the sum of the individual contributions of immunoglobulin and non-immunoglobulin defence proteins such as lactoferrin, lactoperoxidase, lysozyme and peptides. This may be due to the synergistic activity of naturally occurring proteins and oligopeptides, in addition

to peptides generated from inactive protein precursors (Gobbetti *et al*., 2004). It has been proved that milk proteins can also act as antimicrobial-peptide precursors, and in this way might enhance the organism's natural defences against invading pathogens. Consequently, food proteins can be considered as components of nutritional immunity (Pellegrini, 2003).

Antibacterial peptides derived from lactoferrin have attracted much attention during the last decade. The first report that demonstrated the enzymatic release of antibacterial peptides with more potent antimicrobial activity than the precursor protein dates from 1991 (Tomita *et al*., 1991). Shortly afterwards, the antibacterial domains of bovine lactoferrin f(17–41) and human lactoferrin f(1–47), called respectively bovine and human lactoferricin, were purified and identified (Bellamy *et al*., 1992). These peptides showed potent antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria (Wakabayashi *et al*., 2003). Hydrolysis of caprine and ovine lactoferrin by pepsin resulted in antibacterial hydrolysates, and a homologous peptide to lactoferricin, corresponding to fragment f(14–42), was identified in the caprine lactoferrin hydrolysate. The region corresponding to the lactoferricin within the sequence of ovine lactoferrin was hydrolysed by the action of pepsin, and hence the activity observed in the ovine lactoferrin hydrolysate could be caused by other protein fragments (Recio & Visser, 2000). In addition to these studies, El-Zahar *et al*. (2004) obtained a peptic hydrolysate of ovine ALA and BLG that inhibited the growth of *Escherichia coli* HB101, *Bacillus subtilis* Cip5262 and *Staphylococcus aureus* 9973 in a dose-dependent manner, but the peptides responsible for this activity were not identified.

In the same way as whey proteins, caseins are also a source of antimicrobial peptides (López-Expósito & Recio, 2006, 2008). In a preliminary study, an ovine β-casein hydrolysate produced with pepsin, trypsin and chymotrypsin showed inhibition of bioluminescent production by *E. coli* JM103, but the peptides responsible for this activity have not been identified (Gómez-Ruiz *et al*., 2005). Recently, four antibacterial peptides were identified from a pepsin hydrolysate of ovine α_{s2}-casein (López-Expósito *et al*., 2006). The peptides corresponded to α_{s2}-casein f(165–170), f(165–181), f(184–208) and f(203–208), being the fragments f(165–181) and f(184–208) homologous to those previously identified in the bovine protein (Recio & Visser, 1999) (Table 25.6). These peptides showed strong activity against Gram-negative bacteria. Of them, f(165–181) was the most active against all bacteria tested. The peptide corresponding to ovine α_{s2}-casein f(203–208), with sequence PYVRYL, is a good example of a multifunctional peptide because it exhibited not only certain antimicrobial activity but also potent antihypertensive and antioxidant activity (Recio *et al*., 2005; López-Expósito *et al*., 2006). Ovine caseinate hydrolysed with *Bacillus* sp. P7 protease for 3 hours showed antibacterial activity against *Bacillus cereus*, *Corynebacterium fimi* and filamentous fungi (*Penicillium expansum* and *Aspergillus fumigatus*). Because these fungi may cause disease in humans and plants, ovine casein hydrolysates or derived peptides could be used as food-grade biopreservatives (Correa *et al*., 2011).

Antimicrobial activity has also been found in the water-soluble extract of several Italian cheese varieties, some of them manufactured from ovine milk. Most of the extracts exhibited a large inhibitory spectrum against Gram-positive

Table 25.6. Other bioactive peptides derived from ovine milk proteins.

Peptide fragment	Sequence	Biological activity	Reference
α_{s1}-Casein f(10–21)	GLSPEVLNENLL	Antibacterial	Rizzello *et al*. (2005)
α_{s1}-Casein f(22–30)	RFVVAPFPE	Antibacterial	Rizzello *et al*. (2005)
α_{s1}-Casein f(24–31)	VVAPFPE	Antibacterial	Rizzello *et al*. (2005)
α_{s2}-Casein f(165–170)	LKKISQ	Antibacterial	López-Expósito *et al*. (2006)
α_{s2}-Casein f(165–181)	LKKISQYYQKFAWPQYL	Antibacterial	López-Expósito *et al*. (2006)
α_{s2}-Casein f(184–208)	VDQHQAMKPWTQPKTKAIPYVRYL	Antibacterial	López-Expósito *et al*. (2006)
κ-Casein f(112–116)	KDQDK	Antithrombotic	Qian *et al*. (1995a)
κ-Casein f(163–171)	TAQVTSTEV	Antithrombotic	Qian *et al*. (1995a)
κ-Casein f(165–171)	QVTSTEV	Antithrombotic	Qian *et al*. (1995a)
κ-Casein f(98–105)	HPHPHLSF	Antioxidant	Gómez-Ruiz *et al*. (2008)
Lactoferrin f(17–41)	ATKCFQWQRNMRKVRGPPVSCIKRD	Antibacterial	Vorland *et al*. (1998)
Lactoferrin f(14–42)	QPEATKCFQWQRNMRKVRGPPVSCIKRDS	Antibacterial	Recio & Visser (2000)

and Gram-negative microorganisms, including potentially pathogenic bacteria of clinical interest. Some peptide sequences were identified in these extracts and some of them showed correspondence or high homology with previously described antimicrobial peptides (Table 25.6) (Rizzello *et al*., 2005).

25.3.1.3 Other biological activities of peptides from ovine proteins

It has been reported that milk proteins of ovine origin are a source of peptides with other biological activities. For instance, κ-CMP is one of the main components of whey and is obtained as a by-product in cheesemaking. The κ-CMPs from several animal species have been reported as a good source of antithrombotic peptides. Qian *et al*. (1995a) found two very active sequences with inhibitory activity on human platelet aggregation induced by thrombin and collagen after hydrolysing ovine κ-CMP with trypsin (Table 25.6). Furthermore, bovine, ovine and caprine κ-CMPs and their hydrolysates with trypsin were found to be inhibitors of human platelet aggregation (Manso *et al*., 2002). In this work, the hydrolysate obtained from ovine κ-CMP showed the strongest effect, but the peptides responsible for this activity were not identified. Similarly, a chromatographic fraction from a peptic hydrolysate of ovine lactoferrin has also shown inhibitory activity on platelet aggregation (Qian *et al*., 1995b).

Several studies have focused their attention on the identification of peptides derived from caseins and whey proteins of bovine origin with potent antioxidant activity which act by different mechanisms. These peptides were released from the precursor proteins by enzymatic hydrolysis and milk fermentation. More recently, the potential of different ovine casein fractions and their hydrolysates to exert antioxidant activity has also been studied (Gómez-Ruiz *et al*., 2008). Of special interest was the identification of a κ-casein fragment, HPHPHLSF, that resulted in a potent inhibitor of linoleic acid oxidation with similar activity to that obtained with the synthetic antioxidant BHT. Other peptides, such as β-casein f(191–194), with antioxidant activity have been identified in a cheese-like system of milk coagulated with *Cynara cardunculus* (Silva *et al*., 2006). Recently, ovine caseinate has been hydrolysed with a protease preparation of *Bacillus* sp. P7 and displayed antioxidant, antihypertensive and antimicrobial properties (Correa *et al*., 2011).

Currently, the field of application of these bioactive peptides and the study of other biological activities is receiving special attention. Recently, Tulipano *et al*. (2011, 2012) have reported that peptides derived from bovine, ovine and caprine ALA, like the tripeptide with sequence IPA, can be regarded as moderate inhibitors of dipeptidyl-dipeptidase-4. Other peptides have shown immunomodulatory effects, such as a proline-rich polypeptide also known as colostrinin, which was originally found as a fraction accompanying ovine immunoglobulins. This peptide has been found to promote T-cell maturation and pro-cognitive functions in experimental animal models, indicating the prevention of pathological processes in the central nervous system. In humans, it has been demonstrated that the therapeutic benefit of colostrinin in patients with Alzheimer's disease is a consequence of delayed progression of the disease (Zimecki, 2008). Recently, Rodríguez Saint-Jean *et al*. (2012) showed that hydrolysates from bovine and ovine total casein and their isolated fractions of bovine α_{s2}-casein hydrolysate exert antiviral activities against salmonid fish viruses.

Therefore, although research on bioactive peptides has mainly focused on bovine milk proteins, paying less attention to milk from other origins, such as ovine milk, these findings justify further studies on this species. In addition, given the high homology among the sequences of bovine, ovine and caprine milk proteins, it can be predicted that the peptides reported as bioactive agents and released from bovine proteins are also within sheep and goat milk proteins.

25.4 CARBOHYDRATES

Lactose is the major carbohydrate in ovine milk and is composed of glucose and galactose bonded by a $\beta 1 \rightarrow 4$ glycosidic linkage. The lactose content in sheep milk is similar to that in bovine milk, while the fat and protein contents are considerably higher (Ramos & Juárez, 2011). Lactose is a valuable nutrient because it favours intestinal absorption of calcium, magnesium and phosphorus, and the utilisation of vitamin C.

Carbohydrates other than lactose, such as glycopeptides, glycoproteins and oligosaccharides, are also found in ovine milk. Nowadays, milk oligosaccharides are thought to be beneficial for the human milk-fed infant with regard to their prebiotic and anti-infective properties. Recent studies have suggested that human milk oligosaccharides have the potential to modulate the gut flora, to affect different gastrointestinal activities and to influence inflammatory processes (Kunz & Rudloff, 2006). Milk from other mammals also contains oligosaccharides but they are found in lower amounts than in human milk. Recently, it was found that oligosaccharides in caprine milk ranged from 250 to 300 mg/L (Martínez-Férez *et al*., 2006). This represents four to five times the amount of oligosaccharides in bovine milk. The concentration of oligosaccharides in ovine milk ranges from 20 to 30 mg/L, although colostrum, as in other mammalian species, contains considerably higher concentrations. The chemical structures of oligosaccharides

from ovine colostrum have been described by Urashima *et al*. (1989). Three neutral milk oligosaccharides, isomers of galactosyllactose, Gal(α1→3)Gal(β1→4)Glc, Gal(β1→3)Gal(β1→4)Glc, and Gal(β1→6)Gal(β1→4)Glc, have been identified. Three acid milk oligosaccharides from ovine colostrum have been isolated and identified by ^{1}H-NMR (Nakamura *et al*., 1998). These acid milk oligosaccharides contain sialic acid. Sialic acid is a general name for *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). The sialyl oligosaccharides described are Neu5Ac(α2→3)Gal(β1→4)Glc, Neu5Gc(α2→3)Gal(β1→4)Glc, and Neu5Gc(α2→6)Gal(β1→4)Glc. More recently, Martínez-Férez *et al*. (2006) also identified oligosaccharides with similar structures in sheep milk.

These components could be of interest because it has been suggested that sialic acid present in milk oligosacharides promotes the development of the infant's brain (Nakamura & Urashima, 2004). In addition, it has been shown that sialic acid-containing oligosacharides reduce the adhesion of leucocytes to endothelial cells, an indication of an immune regulatory effect of certain human milk oligosaccharides (Kunz & Rudloff, 2008).

25.5 MINERALS

There are about 20 minerals that are considered to be nutritionally essential for humans (Na, K, Cl, Ca, Mg, P, Fe, Cu, Zn, Mn, Se, I, Cr, Co, Mb, F, As, Ni, Si and B). Milk and dairy products can make an important contribution to the daily intake of some of them, especially Ca and P. Detailed descriptions of the biochemical role of these essential minerals and trace elements as well as of the nutritional significance of milk as a source of these micronutrients have been profusely discussed elsewhere (Renner *et al*., 1989; Flynn & Cashman, 1997; Cashman, 2002a, b; Cosentino *et al*., 2010) and are not dealt with in this chapter.

The minerals in sheep milk have not been as extensively studied as those in bovine milk, even though they may be of nutritional and health interest. Sheep milk has around 0.9% total minerals or ash compared to 0.7% in cow or goat milk (Juárez & Ramos, 1986; Mayer & Fiechter, 2012). The most abundant elements are Ca, P, K, Na and Mg whilst Zn, Fe, Cu and Mn are the more remarkable trace elements. Representative values for the average mineral content of milk are presented in Table 25.7. The levels of Ca, P, Mg, Zn, Fe and Cu are higher in sheep than in cow milk, while the opposite appears to be the case for K. The contents of macrominerals and trace elements in other sheep dairy products have been presented elsewhere (Martín-Hernández *et al*., 1992; Coni *et al*., 1999). In general, the mineral content of sheep milk seems to vary much more than that of cow milk and is not constant, being influenced by a number of factors such as stage of lactation, nutritional status of the animal, and environmental and genetic factors due to feeding differences and seasonal variations (Polychroniadou & Vafopoulou, 1985; Rincón *et al*., 1994).

The chemical form in which a macromineral or trace element is found in milk is important because it may influence intestinal absorption and utilisation (the process of transport, cellular assimilation and conversion into a biologically active form) and thus bioavailiability. The salt balance in sheep milk is also interesting as a contribution to knowledge of the nutritional characteristics of these types of milk, and to the retention of these elements in the curd during cheesemaking. Since sheep milk is mainly used in cheesemaking and most of the soluble elements are lost in the whey during manufacture, knowledge of element distributions would allow to evaluate the influence of milk composition on the mineral content in cheese.

Na, K and Cl in milk are almost entirely soluble and fully available in the whey. Ca, Mg and P in sheep milk are associated in different proportions with the colloidal suspension of casein micelles. Because of these associations, these minerals are partly retained in the curd during cheesemaking. In samples from different herds on farms in Spain, the percentages of Ca, Mg and P in the soluble phase of sheep milk were 21, 56 and 35%, respectively (De la Fuente *et al*., 1997), within the range of variation reported in other countries (Polychroniadou & Vafopoulou, 1986; Pellegrini *et al*., 1994). Although the proportions of P and Mg linked to casein were, in general terms, higher than in cow milk, the most striking aspect of these findings is the distribution of Ca. Percentages of Ca in the soluble phase are lower than in milk from other ruminants and thereby higher levels of this element can potentially be incorporated into the curds.

Data on the distribution of trace elements in sheep milk are scarce. It appears that a large proportion (up to 90%) of Zn and Mn is found in the micellar fraction (Kiely *et al*., 1992; Shen *et al*., 1995; De la Fuente *et al*., 1997), presumably casein, as occurs in cow milk, and is the principal zinc-binding ligand in these species. The distribution of Fe and Cu differed more. Along with Cu, Fe is the most abundant microelement in the soluble phase. This fraction contains 29% and 33% of total Fe and Cu, respectively (De la Fuente *et al*., 1997). Additionally, of all the elements considered here, Fe is probably the one that is bound in the highest proportion to the lipid fraction. Of the remaining trace elements, it is noteworthy that Se availability in sheep milk appears to be significantly lower than in human, bovine and caprine milks (Shen *et al*., 1996).

The content of Ca and P in cheese is higher than that in milk, four to five times higher in fresh cheeses, seven to

Table 25.7. Values for vitamins (per 100 g) and minerals (per litre) of sheep, goat and cow milk.

	Sheep			Goat	Cow
	Park *et al.* (2007)	**Park & Guo (2006)**	**Raynal-Ljutovac *et al.* (2008)**	**Raynal-Ljutovac *et al.* (2008)**	**Raynal-Ljutovac *et al.* (2008)**
Vitamins					
Vitamin A (μg)	44	83	80	40	40
Thiamin B_1 (μg)	80	80	80	50	40
Riboflavin B_2 (mg)	0.38	0.32	0.35	0.14	0.17
Niacin B_3 (mg)	0.42	0.41	0.42	0.20	0.09
Pantothenic acid B_5 (mg)	0.41	0.45	0.41	0.31	0.34
Pyridoxine B_6 (mg)	—	0.08	0.08	0.05	0.04
Biotin B_8 (μg)	0.93	2.50	—	2.00	2.00
Folic acid B_9 (μg)	5.00	5.00	5.00	1.00	5.30
Cobalamin B_{12} (μg)	—	0.60	0.71	0.06	0.35
Vitamin C (mg)	4.16	—	5.00	1.30	1.00
Vitamin D (μg)	0.18	0.18	0.18	0.06	0.08
Vitamin E (μg)	—	110	110	40	110
Minerals					
Ca (g)	1.93	—	1.95–2.00	1.26	1.20
Mg (g)	0.18	—	0.18–0.21	0.13	0.11
Na (g)	0.44	—	0.44–0.58	0.38	0.45
K (g)	1.36	—	1.36–1.40	1.90	1.50
P (g)	1.58	—	1.24–1.58	0.97	0.92
Fe (mg)	0.80	—	0.72–1.22	0.55	0.46
Cu (mg)	0.40	—	0.40–0.68	0.30	0.22
Zn (mg)	5.70	—	5.20–7.47	3.40	3.80
Cl (g)	1.60	—	1.10–1.20	1.60	1.10
Mn (μg)	70.0	—	53–90	80	60
I (mg)	0.20	—	0.10	0.08	0.07

Source: based on data from Park *et al*. (2007), Park & Guo (2006) and Raynal-Ljutovac *et al*. (2008).

eight times higher in semi-hard cheeses, and ten times higher in hard cheeses. The bioavailability of Ca in cheese is comparable to that from milk and is not affected by the ripening process (Ramos & Juárez, 2011).

25.6 VITAMINS

Bibliographical data on the vitamin content of sheep milk are given in Table 25.7. Most of the known vitamins are contained in ovine milk and for some of them this foodstuff is a rich source. Daily riboflavin (B_2) requirements, for instance, would be completely covered by drinking just two cups of sheep milk without eating anything else (Haenlein, 2001). As drinking sheep milk is not widespread, more likely two cups of sheep milk yoghurt would meet those daily requirements, or the milk equivalent in 90 g of sheep cheese. From the literature (Park & Guo, 2006; Park *et al*., 2007; Raynal-Ljutovac *et al*., 2008; Ramos & Juárez, 2011) the conclusion can be drawn that sheep milk is richer than cow milk in most of the vitamins (Table 25.7). However, documented research data on the vitamins of sheep milk are too sparse to offer a definitive picture.

25.7 SHEEP MILK PRODUCTS

Practically all sheep milk is used to make cheeses. Cheese represents one of the most nutritionally complete foods and has the potential to make an important contribution to the health of populations (Walther *et al*., 2008). It provides a rich source of important nutrients, such as proteins, fat, vitamins and minerals (Table 25.8). Calcium, which is present in large quantities in cheese, has been shown to have a positive effect on various disorders (hypertension, osteoporosis, obesity and dental caries). Beside calcium, other constituents with potentially positive effects (e.g. bioactive peptides, CLA, vitamin A, folic acid and cobalamin) are also found (Ash & Wilbey, 2010). The inclusion of cheese

Table 25.8. Chemical composition of cheeses made from sheep milk.

Country	Name	Type of cheese	Fat content (% total solids)	Protein (%)	Ca (%)	P (%)	Vitamin A (μg/100 g)	Vitamin B_9 (folic acid) (μg/100 g)	Vitamin B_{12} (cobalamin) (μg/100 g)
France	Roquefort	Blue-veined	50	21	0.62	0.42	295	45	0.4
Greece	Feta	Brined	40	18	0.65	0.40	220	23	1.1
Italy	Pecorino	Hard	42	29	0.40	0.42	NR	NR	NR
Portugal	Serra da Estrela	Semi-hard	56	20	0.65	0.53	NR	NR	NR
Spain	Manchego	Semi-hard	51	23	0.68	0.54	357	20	1.5
Spain	Burgos	Fresh	54	16	0.61	0.38	320	9	0.5
Greece	Manouri	Whey	82	11	NR	NR	NR	NR	NR

NR, not reported.

Sources: based on data from Marcos *et al*. (1983), Marcos & Esteban (1993), Haenlein (1998), O'Connor & O'Brien (2000) and Ramos & Juárez (2011).

as part of a balanced diet is more likely to assist rather than hinder well-being, particularly in groups who may be consuming inadequate quantities of calcium in their diets or who are lactose intolerant.

There are six categories of sheep milk cheese based on the technology used: fresh, white brined, blue-veined, semi-hard, hard and whey cheeses (Ramos & Juárez, 2011). Roquefort is a French blue-veined cheese made from raw milk inoculated with *Penicillium roqueforti* spores. It displays high levels of proteolysis and lipolysis after 5 months of ripening. Feta cheese is a white brined cheese originally from Greece, and is one of the most popular internationally. Traditionally it is made from raw milk and is ripened in barrels with brine (6–8% NaCl) for around 1 month at 8–10°C; it is then kept chilled for at least 2 months prior to consumption. It is now made mainly from pasteurised milk. Pecorino is an Italian hard cheese made with raw or pasteurised milk, in most cases coagulated with lamb or kid rennet paste. Proteolysis is moderate but there is intense lipolysis due to the presence of pregastric esterases in the traditional curd pastes used.

The most characteristic Spanish semi-hard sheep milk cheese is Manchego. This is generally ripened for 6 months or longer. Proteolysis is medium/high, while lipolysis is low. Some semi-hard sheep milk cheeses are made in Portugal using vegetable rennet extracted from the flower of the chard *Cynara cardunculus*. Examples include Serra da Estrela. These are commercialised after relatively short ripening (30–60 days), but proteolysis levels are high due to the strong proteolytic activity in the curds. Spain produces popular fresh cheeses as Burgos. This is now made from pasteurised milk with animal rennet, but without starter cultures. It must be kept in chilled storage and consumed in less than 6 days.

Finally, in various countries the whey from sheep milk cheesemaking is used on its own or mixed with milk to manufacture products of high nutritional value (Pintado *et al*., 2001). The whey contains mainly water-soluble proteins (~1%), fat (<1%), lactose, minerals, non-nitrogen substances and vitamins. These whey cheeses are the result of heat-induced coagulation of the whey protein (which occludes fat). The best-known whey cheeses are Manouri and Mizithra from Greece, Requesón from Spain, Ricotta from Italy and Gjetost from Norway.

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26
Camel Milk

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26.1 INTRODUCTION

The camel family Camelidae comprises the Bactrian camel (*Camelus bactrianus ferus*), dromedary camel (*Camelus dromedarius*), llama (*Lama glama*), guanaco (*Lama guanicoe*), alpaca (*Vicugna pacos*), and vicuña (*Vicugna vicugna*). Bactrian (two-humped) and dromedary (one-humped) camels were domesticated between 4000 and 5000 years ago in Central Asia and the Arabian Peninsula, respectively, and they have been important sources of energy as well as means of transportation for nomads living in arid and semi-arid areas. Bactrian camels are adapted to cold environments in the Gobi desert and surrounding areas (Fig. 26.1) and in Kazakhstan, Asia Minor, and southern Russia, and their estimated population size is approximately one-tenth that of dromedaries. The number of Bactrian camels in Mongolia has declined from 750 000 to 200 000 over the past 50 years (Dubach *et al*., 2007). The presence of wild Bactrian camels has been confirmed in and around the Gobi desert area in Mongolia and in Inner Mongolia in China. Despite protection measures instituted by the Mongolian and Chinese governments, the population has been continuously decreasing (from 500–600 in the 1980s to 350 in 2004 in Mongolia; 600 surviving in 2004 in China), due to the low survival rate of calves, the threat of hunting, the settling of oases by pastoralists, hybridization with domestic camels, and climate change (Tulgat & Schaller, 1992; Reading *et al*., 1999; Dubach *et al*., 2007; Hare, 2008). Wild Bactrian camels are currently listed under "critically endangered" on the IUCN Red List of Threatened Species. In contrast, dromedary camels are well adapted to hot and dry environments, and are widely distributed throughout the Middle East, North and East Africa, south west Asia, and Australia, with increasing numbers of more than 15 million head (Al haj & Al Kanhal, 2010). It is common that Bactrian and dromedary camels cohabit in Kazakhstan leading to hybridization of the species.

Camel meat is not as commonly consumed as other meat, but it is certainly an important source of nutrition especially in areas with severe environmental conditions where people are unable to obtain meat from beef. In fact, camels are good suppliers of meat; the carcass of male and female camels can weigh up to 400–650 and 250–350 kg, respectively (Yagil, 1982). Being adapted to a cold climate, the body of Bactrian camels is covered with hair that provides good-quality material for wool fabrics. The highest yield of hair is attained when the animal is 5 years old, with an average of 8–9 and 5.2–5.5 kg for male and female camels, respectively (Dubach *et al*., 2007). Camels can travel 150 km (93.2 miles) in 15–20 hours and run at 65 km/hour (40 mph) in short bursts and at 40 km/hour (25 mph) at a constant speed. Therefore camels are widely used as vehicles for travel and racing.

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Figure 26.1. Domesticated Bactrian camel in Gobi desert area. (Left) A nursing camel. (Right) A camel being milked by a nomad woman. Photos taken by the author in March 2009 in Haruult and Zagiin-Us, Ulziit soum, Dundgovi aimag in Mongolia (about 260 km south of Ulaanbaatar). For a color version of this figure, see Plate 26.1.

Like cows, camels have a four-quartered udder. The lactation period varies from 9 to 18 months, and calculated milk yields range from 735 to 10 675 kg per 305 days, depending on the individual, breed, stage of lactation, feeding, and management conditions (Yagil, 1982). The calorie content of camel milk (665 kcal/L) is similar to that of cow milk (701 kcal/L) (El-Agamy, 2009). Although the yield of milk from camels is not as high and stable as that obtained from cows, recent reports have shown that camel milk contains all the essential nutrients found in bovine milk (El-Agamy *et al*., 1998) and that camel milk contains potentially beneficial compounds for human health, such as anticarcinogenic (Magjeed, 2005), antidiabetic (Agrawal *et al*., 2007; Hamad *et al*., 2011), and antihypertensive (Quan *et al*., 2008) substances. These findings encourage more consumption of camel milk and its dairy products. A potential value of camel milk as a substitute for cow milk has been reported for children who are allergic to cow milk, because no cross-reactivity was observed to cow milk proteins when specific antisera of camel milk proteins were applied in Western blot analysis (El-Agamy *et al*., 2009).

Several comprehensive reviews and book chapters deal with the subject of camel milk (El-Agamy, 2006, 2009; Al haj & Al Kanhal, 2010; Farah, 2011). Most of them focus mainly on dromedary camels, in proportion to their numbers, whereas overviews on Bactrian camels are scarce. In this chapter, therefore, studies on Bactrian camel milk have been covered to shed light on its nutritional and economical importance in comparison with dromedary camel milk.

26.2 CAMEL MILK PRODUCTION AND UTILIZATION WORLDWIDE

26.2.1 Camel milk production

The FAO/CIRAD/KARKARA workshop estimated that global camel milk output is not less than 5.3 million tonnes, while available worldwide production of camel milk has been assessed at 1.3 million tonnes (FAO, 2008), although it should be taken into account that these values are obtained under pastoral conditions. This probably means that three-fourth or more of camel milk is consumed by nomads in the form of raw milk and dairy products aimed at a long shelf-life. In 2005, Somalia was the country with the highest camel milk production (0.85 million tonnes per year), accounting for more than half of the available camel milk production in the world (Table 26.1). At that time, Bactrian camels domesticated in Mongolia and in Inner Mongolia in China produced only 1.2% of the total camel milk production.

26.2.2 Utilization of Bactrian camel milk

According to the categorization of milk processing systems by Hirata (2008), there are eight groups on the Asian continent: (i) Central Asia North Highland type, (ii) Central Asia Lowland type, (iii) North Asia type, (iv) Tibetan Plateau type, (v) West Asia: Anatolia type, (vi) West Asia: Arab type, (vii) West Asia: Persia type, and (viii) South Asia type. Mongolia and Inner Mongolia (China) belong to the North Asia type, where cream separation and alcohol fermentation techniques have evolved very well. Milk processing in

Table 26.1. Worldwide production of camel milk (tonnes/year).

China	**14 400**
Mongolia	1 000
Algeria	8 100
Chad	22 050
Djibouti	5 900
Eritrea	5 100
Ethiopia	23 500
Kenya	25 200
Libyan Arab Jamahiriya	2 000
Mali	55 200
Mauritania	22 000
Morocco	3 800
Niger	10 800
Quatar	9 900
Saudi Arabia	90 000
Somalia	850 000
Sudan	82 250
Tunisia	1 000
United Arab Emirates	39 350
Yemen	13 600

Source: based on data from FAO (2008).

this area is generally simple and quick, with three main processing flows as shown in Fig. 26.2.

Initially, raw milk (*inge suu* in the case of camel; note that domestic names of dairy products have been italicized), directly or following boiling (the boiled milk is called *tsagaa*), is fermented at room temperature to make acidified milk (Fig. 26.3), or alcoholic fermentation is performed to make the alcoholic beverage *airag* (*khoormog* or *tsegee* in the case of camel milk) in a fermented milk processing flow (Fig. 26.2a). Cultured camel milk product similar to yogurt is called *tarag*. Cheese (*aaruul*) is made by dehydration of both acidified milk and *airag*, and spirit (*shimiin arhi*) is produced by distillation of *airag*. The average fat and protein content (37.1 and 31.7%, respectively) in camel *aaruul* are higher than in cow *aaruul* (16.0 vs. 24.8%), and the higher fat content in camel *aaruul* seems to cause its brittleness (Ishii & Samejima, 2006). The same trend found in the fat and protein content of *aaruul* is naturally seen among alcoholic beverages made from camel and cow milks (Ishii & Samejima, 2006). Cheese is made not only by fermentation but also by addition of strong acid milk to the raw milk (Fig. 26.2b). The curd made from camel milk is called *aarts*.

It is characteristic of the North Asia type of milk processing system that people who live in this area make alcoholic beverages from the skim milk of all five domestic animals: cow, sheep, goat, horse, and camel (Fig. 26.2c). A fresh cheese-like product (*byaslag*) is also made from casein extracted from the skim milk. *Eezgii* is made by boiling down the skim milk. Another feature of this cream separation processing flow is the post-processing method of the separated cream. People make butter by churning in Inner Mongolia (China), whereas in Mongolia people generally directly refine butter oil (*shar tos*) from the cream (*orom*) (Hirata, 2008). In some cases butter (*tos*) is made by churning of fermented camel milk, *khoormog* or *tsegee*, in Mongolia.

Mongolian nomads make *butsalgaa* by mixing *airag* and fermented camel milk. *Butsalgaa* has reduced sourness and rich taste. It is believed that this type of beverage has an anti-swelling and therapeutic effect on intestinal and kidney diseases (Indra & Erdenbaatar, 1998).

26.2.3 Utilization of dromedary camel milk

In contrast to the culture of camel milk processing in Mongolia and in Inner Mongolia (China), no alcoholic fermentation occurs in areas where dromedary camels are used, mainly because of different climatic conditions. Although it seems to be difficult to develop milk preservation techniques, other than butter or butter oil production, under the hot and dry desert environments, a variety of traditional dromedary camel milk products do indeed exist, such as dried curd from fermented milk (*oggt*) in Saudi Arabia, fermented milk (*susa*) in East Africa, clarified fat (*ghee*) in Somalia, and fermented camel milks of *shubat* in Kazakhstan (Konuspayeva *et al*., 2004), *suusac* in Kenya (Lore *et al*., 2005), *gariss* in Sudan (Sulieman *et al*., 2006), and butter of *shmen* in Algeria (Kacem & Karam, 2006).

Although dromedary camel milk was proposed as suitable for drinking only relatively recently (Yagil *et al*., 1984), several attempts at milk processing have been performed, including butter (Farah *et al*., 1989), ice cream (Abu-Lehia *et al*., 1989), soft cheese (Mehaia, 1993; El Zubeir & Jabreel, 2008), and yogurt (Abu-Tarboush, 1996; Abu-Tarboush *et al*., 1998; Hashim *et al*., 2009), under laboratory conditions. Moreover, a company in the United Arab Emirates has recently started commercial production of chocolates containing camel milk.

26.2.4 Utilization of camel milk in Australia

Thousands of camels were imported mainly from India for transportation purposes in nineteenth-century Australia. They were used to pioneer the dry and arid interior, to

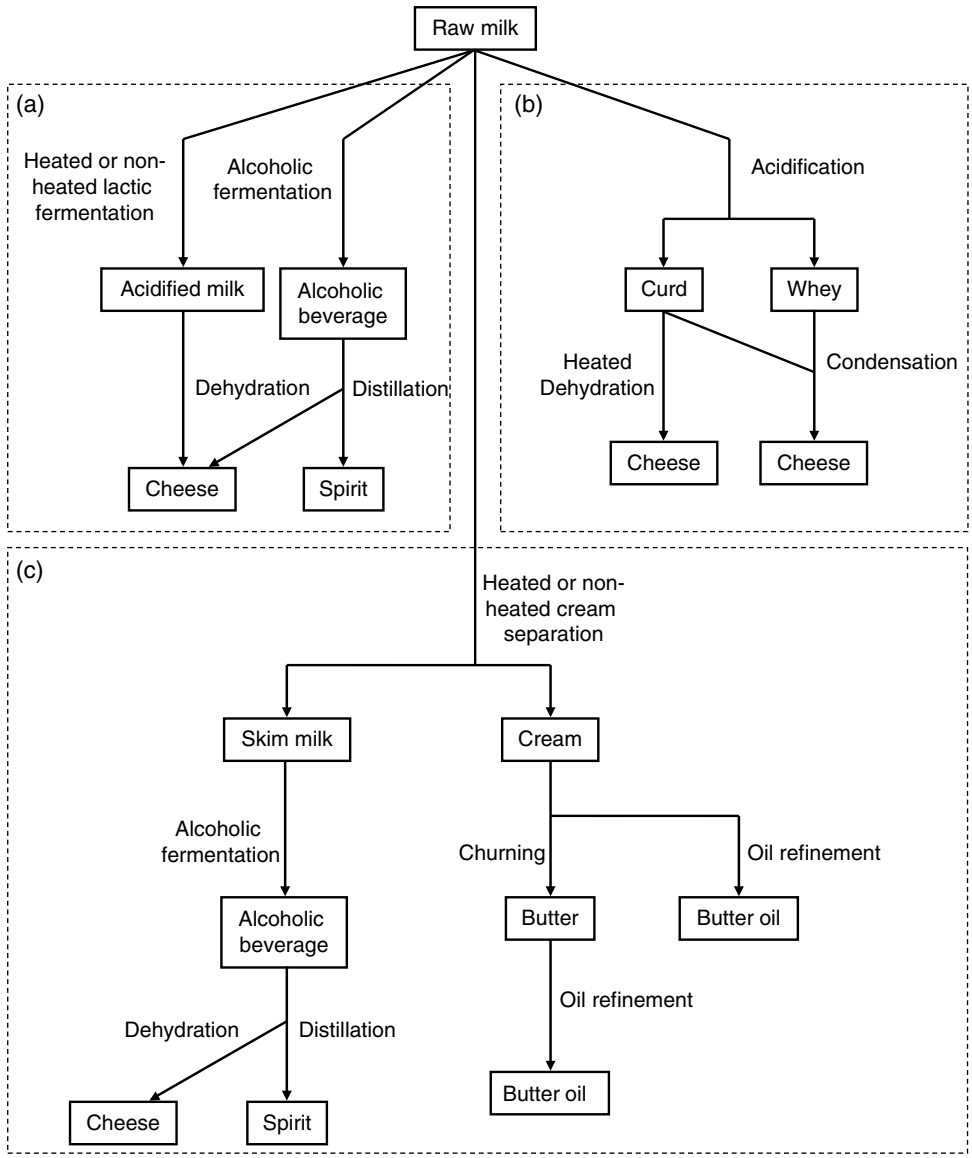


Figure 26.2. Schematic view of milk processing in Mongolia and in Inner Mongolia (China). Modified with permission from Hirata (2008).

construct rail and telegraph lines, and to supply goods to remote mines and settlements. As they were replaced by motorized transportation, domesticated camels were released and became feral, spreading across arid and semi-arid areas of the Northern Territory, Western Australia and South Australia, and into parts of Queensland. According to Australian government statistics, the population of feral camels in Australia was estimated in 2004 at more than 500 000 and is increasing, doubling about every 9 years (Anon., 2009). This has caused significant economic losses and damage to the ecological system, and hence the Australian government controls the number of feral camels. In this context, there are attempts to harvest and export live camels in order to control their population in the arid central regions of Australia. Several enterprises that produce camel milk products (e.g., powder, cosmetics, and soap) are also emerging in Australia, but the volumes of this trade are unclear.



Figure 26.3. A cask for making acidified camel milk. Photo taken by the author in March 2009 in the same location as for Figure 26.1. For a color version of this figure, see Plate 26.2.

Table 26.2. Mean concentrations (mg/100 g) of minerals in camel, cow, and human milk.

	Camel			
	Bactrian[1]	**Dromedary**[2]	**Cow**[3]	**Human**[3]
Ca	109	114	120	31
Mg	11	11	12	2.7
Na	46	59	51	12
K	137	156	137	64
P	120	55	65	7.8
Zn	0.65	0.59	0.4	0.15
Mn	ND	0.005	0.003	0.001
Fe	0.21	0.29	0.03	0.047

ND, no data.

Sources:

[1]Zhang *et al*. (2005), Faye *et al*. (2008) and Jirimutu *et al*. (2010).

[2]Gnan & Sheriha (1986), Abu-Lehia (1987), Hassan *et al*. (1987), Elamin & Wilcox (1992), Mehaia *et al*. (1995), Haddadin *et al*. (2008) and Konuspayeva *et al*. (2010).

[3]Dorea (2000), Yamawaki *et al*. (2005), Cashman (2011) and Gaucheron (2011).

26.3 CAMEL MILK COMPONENTS AND THEIR NUTRITIONAL ASPECTS

26.3.1 Mineral salts and vitamins

The mean concentrations of copper, magnesium, sodium, potassium, phosphorus, zinc, manganese, and iron in camel, cow, and human milk are summarized in Table 26.2. Variations in the major mineral contents are attributed to analytical method, breed, feeding, stage of lactation, and water intake (Elamin & Wilcox, 1992; Mehaia *et al*., 1995; Zhang *et al*., 2005; Haddadin *et al*., 2008; Konuspayeva *et al*., 2010). In general, the concentrations of the major minerals in the milk of camels are rather similar to those of cows rather than humans. Phosphorus is in higher concentrations in Bactrian than in dromedary milk (Faye *et al*., 2008). It is important to remember that the ratio of calcium to phosphate in milk given to human newborn infants is appropriate in order to avoid hyperphosphatemia and hypocalcemia (Gittleman & Pincus, 1951; El-Agamy, 2006). Camel milk is a good source of iron and can support rapid growth in infancy and help avoid iron-deficiency anemia. Dromedary camel milk is also rich in chloride as a result of their forage diet, e.g., *Atriplex* and *Acacia* (Khaskheli *et al*., 2005). It is known that the antimicrobial activity of lactoferrin is enhanced by low levels of citrate, so the lower citrate concentration in camel milk (128 mg/dL) than in cow milk (160 mg/dL) may be advantageous (El-Agamy, 2006).

The mean concentrations of vitamins in camel, cow, and human milk are summarized in Table 26.3. Camel milk is obviously a rich source of vitamins B_1, B_2, B_6, B_{12}, and C. In particular, higher concentrations of vitamin C in camel milk than in cow or human milks are beneficial for nutrition. Although there are large variations in the vitamin concentrations of camel milk owing to breed, feed, stage of lactation, and so on, it is likely that concentrations of vitamins A, D, and E in milk are higher in Bactrian than in dromedary camels (Zhang *et al*., 2005).

26.3.2 Lipids

The fat content of camel milk ranges from 1.2 to 6.4% (Konuspayeva *et al*., 2009), which is comparable to that of cow milk. However, the content of short-chain fatty acids

Table 26.3. Mean concentrations (mg/kg) of vitamins in camel, cow, and human milk.

	Camel			
	Bactrian[1]	**Dromedary**[2]	**Cow**[3]	**Human**[4]
A	0.97	0.21	0.28	0.55
B_1 (thiamin)	0.12	0.41	0.59	0.15
B_2 (riboflavin)	1.2	1.1	1.6	0.38
B_3 (niacin)	ND	0.78	0.7	1.7
B_5 (pantothenic acid)	ND	2.3	3.8	2.7
B_6 (pyridoxine)	0.54	0.54	0.5	0.14
B_7 (biotin)	ND	ND	0.04	0.01
B_9 (folic acid)	ND	0.0046	0.055	0.042
B_{12}	ND	0.0053	0.009	0.0005
C	103	140	13	40
D	0.017	0.003	0.009	0.014
E	1.5	0.18	0.6	8

ND, no data.

Sources:

[1] Zhang *et al*. (2005) and Faye *et al*. (2008).

[2] Sawaya *et al*. (1984), Mohamed *et al*. (2005), Faye *et al*. (2008), Haddadin *et al*. (2008) and Konuspayeva *et al*. (2010).

[3] Hill & Morrissey (2011); Morrissey & Hill (2011a,2011b), Nohr *et al*. (2011a–g), Sauvant *et al*. (2011), van Staveren & de Groot (2011) and Witthöft (2011).

[4] Lakdawala & Widdowson (1977), Cooperman *et al*. (1982), Byerley & Kirksey (1985), Haskell & Brown (1999), Korchazhkina *et al*. (2006) and Nohr *et al*. (2011a–g).

(Abu-Lehia, 1989) and carotene (Stahl *et al*., 2006) is lower in dromedary camel milk than in the milk of cows. The ratio of cholesteryl ester fatty acids in camel milk (52%) is slightly lower than in cow milk (58%), palmitic acid being the predominant fatty acid in both (Gorban & Izzeldin, 1999). The content of medium-chain fatty acids such as pelargonic acid (C9:0) and decanoic acid (C10:1) are higher in camel milk than in cow milk (Gorban & Izzeldin, 1999). A detailed analysis of the distribution patterns of triacylglycerols in dromedary camel milk was performed using electrospray ionization tandem mass spectrometry (Haddad *et al*., 2011). This revealed that at least one of the four major fatty acids (C14:0, C16:0, C18:0, C18:1) represented 74% of the total fatty acids among the 99% of quantified triacylglycerols and that at least one unsaturated fatty acid was among the 75% of those fatty acids. Dreiucker and Vetter (2011) have recently reported a comparative analysis of fatty acid patterns of camel, moose, cow, and human milk using a combination of silver ion solid phase extraction and GC/MS. It was revealed that the contents of *iso* and *anteiso* fatty acids were highest in camel milk. Camel milk is generally considered a rich source of the long-chain and unsaturated fatty acids that may lower human serum lipids; however, significant regional variations have been observed in the distribution of milk fatty acids in Jordan (Ereifej *et al*., 2011) and Kazakhstan (Konuspayeva *et al*., 2008). Konuspayeva *et al*. (2008) have also reported that dromedary camel milk has a higher proportion of C17:0 *iso* and C18:1 fatty acids than Bactrian camel milk.

26.3.3 Carbohydrates

The major carbohydrate in milk is lactose, Gal($\beta 1 \rightarrow 4$) Glc, accounting for 2.4–5.8% of total solids in Bactrian and dromedary camel milks (Konuspayeva *et al*., 2009). The average concentration of lactose in Alxa Bactrian camel milk has been reported to be 4.44% (Zhang *et al*., 2005). These values are similar to those of cow milk (4–5%) but less than in human milk (about 7%). The lactose concentration in camel mature milk is rather constant during the different stages of lactation (Hassan *et al*., 1987; Zhang *et al*., 2005), but dehydration evoked by drought conditions leads to decreases in lactose content to 2.9% (Yagil & Etzion, 1980).

Table 26.4. Oligosaccharides found in Bactrian camel colostrum and mature milk.

Structure	Common name
Colostrum	
Neutral oligosaccharides	
Gal(β1→4)[Fuc(α1→3)]Glc	3-Fucosyl lactose
Gal(β1→3)Gal(β1→4)Glc	3′-Galactosyl lactose
Gal(β1→6)Gal(β1→4)Glc	6′-Galactosyl lactose
Acidic oligosaccharides	
Neu5Ac(α2→3)Gal(β1→4)Glc	3′-Sialyl lactose
Neu5Ac(α2→6)Gal(β1→4)Glc	6′-Sialyl lactose
Neu5Ac(α2→3)Gal(β1→3)Gal(β1→4)Glc	(α2→3) Sialyl 3′-galactosyl lactose
Neu5Ac(α2→6)Gal(β1→4)GlcNAc(β1→3)Gal(β1→4)Glc	Sialyllacto-*N*-tetraose c
Gal(β1→4)GlcNAc(β1→6) | Gal(β1→4)Glc | Neu5Ac(α2→3)Gal(β1→3)	Sialyllacto-*N*-novopentaose a
Neu5Ac(α2→6)Gal(β1→4)GlcNAc(β1→6) | Gal(β1→4)Glc | Gal(β1→3)	Sialyllacto-*N*-novopentaose b
Gal(β1→4)GlcNAc(β1→6) | Gal(β1→4)Glc | Neu5Ac(α2→6)Gal(β1→4)GlcNAc(β1→3)	Sialyllacto-*N*-neohexaose
Mature milk	
Neutral oligosaccharides	
Gal(β1→3)Gal(β1→4)Glc	3′-Galactosyl lactose
Gal(β1→4)GlcNAc(β1→6) | Gal(β1→4)Glc | Gal(β1→3)	Lacto-*N*-novopentaose I
Acidic oligosaccharide	
Neu5Ac(α2→3)Gal(β1→4)Glc	3′-Sialyl lactose

Glc, glucose; Gal, galactose; Fuc, fucose; GlcNAc, *N*-acetylglucosamine; Neu5Ac, *N*-acetylneuraminic acid.
Source: reproduced from Fukuda *et al*. (2010), with permission of Elsevier.

In addition to lactose, small amounts of a variety of oligosaccharides, of which the reducing end is usually composed of a lactose unit, are contained in mammalian milk (Urashima *et al*., 2011). Whereas lactose is an important energy source for neonates, milk oligosaccharides are believed to play significant roles in protection against pathogens, promotion of bifidus flora formation, and development of the nervous system (Urashima *et al*., 2009). Using proton nuclear magnetic resonance spectroscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Fukuda *et al*. (2010) identified the presence of 10 oligosaccharides in colostrum and three in mature milk of Bactrian camels (Table 26.4). The core structures of Bactrian camel milk oligosaccharides are lactose, lacto-*N*-neotetraose, lacto-*N*-novopentaose I, and lacto-*N*-neohexaose, known as type II structure; in addition, an acidic oligosaccharide, in which 3′-galactosyl-lactose is the core structure, exists in colostrum. These

characteristics were more similar to bovine milk than human milk (Mariño *et al*., 2011). Camel colostrum may be a better commercial source of sialyl oligosaccharides, because their content is higher than in cow colostrum (Fukuda *et al*., 2010).

Carbohydrates are also present as sugar chains covalently bonded to peptides and proteins in milk. Using five pathogenic bacterial lectins, Zinger-Yosovich *et al*. (2011) observed hemagglutination inhibitory activities in camel milk but these were largely decreased after dialysis, indicating the presence of glycoproteins. Interestingly, the patterns of inhibitory activity among different animal milks were totally different (Zinger-Yosovich *et al*., 2011), indicating that sugar chain structures and glycosylation patterns of milk glycoproteins seem to be fairly diverse.

26.3.4 Proteins

Total protein concentrations in Bactrian and dromedary camel milks are 2.6–4.8 and 2.2–4.9 g/dL, respectively (Konuspayeva *et al*., 2009). As is well known for other mammalian milks, the high protein content of colostrum and its rapid decrease a couple of days after parturition has also been observed in camel milk. Zhang *et al*. (2005) reported that the protein content of Alxa Bactrian camel colostrum was 14.2%, decreasing to 9.63% within the first 12 hours and gradual declining to 3.6% over 90 days. Similarly, Abu-Lehia *et al*. (1989) observed a rapid decrease in protein content, from 13.0% to 5.1%, within the first 24 hours in dromedary camel milk, with a further decline to 4.0% over 10 days. Milk protein content is also affected by breed (Mehaia *et al*., 1995) and by seasonal conditions (Haddadin *et al*., 2008).

26.3.4.1 Caseins

In mammalian milk, caseins (α_{s1}, α_{s2}, β, and κ) are the major proteins, but their total amount and ratio varies among different animal species. Caseins account for about 45, 80, and 70% of total proteins in mature milk of humans, cows, and dromedary camels, respectively. In non-fat milk of Alxa Bactrian camels, the casein fraction was 30.9 ± 2.9% of total protein at 2 hours after birth and increased steadily during lactation, reaching 52.2 ± 0.2% at 90 days after birth (Zhang *et al*., 2005), which is comparable to that found in human milk. The ratio of α_{s1} to α_{s2} to β to κ-casein in cow milk is 37 : 6.1 : 44.2 : 12.7 (Bonizzi *et al*., 2009), whereas the ratio in dromedary camel milk is 22 : 9.5 : 65 : 3.5 (El-Agamy, 2009). A large variation in casein ratios has also been observed among dromedary camel milks collected from different locations in Jordan: α_{s1}/α_{s2}-caseins, 27.02–54.58% of total protein; β-casein, 12.56–33.95%; and κ-casein, undetectable to 8.42% (Ereifej *et al*., 2011). This heterogeneity could stem from a complex combination of factors, including environmental (sampling period and feeding), physiological (stage of lactation and parity), and genetic (breed and polymorphism). Such variations in casein composition ratios in camel milk may cause a larger size distribution of casein micelles in camel milk (ranging from 20 to more than 300 nm diameter) compared with that of cow milk (40–160 nm).

The predominant casein in cow and dromedary camel milks is β-casein; however, its ratio in the total proteins comprising dromedary camel milk is notably higher than that in cow milk and this tendency is similar to that in human milk. Al haj and Al Kanhal (2010) pointed out that the high percentage of β-casein in dromedary camel milk could reflect its higher digestibility rate and lower incidence of allergy in the gut of infants because β-casein is more sensitive to peptic hydrolysis than α_s-caseins. The content of κ-casein is significantly lower in milk of dromedary camels than that of cow milk, which could be a reason for the difficulties in detecting κ-casein using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) with Coomassie Brilliant Blue staining (Farah & Farah-Riesen, 1985). The presence of a κ-like casein in Bactrian and dromedary camel milks was elucidated using alkaline urea PAGE and chymosin treatment (Ochirkhuyag *et al*., 1997), but the qualitative and quantitative differences between them have not yet been unraveled.

26.3.4.2 Whey proteins

As in cow milk, immunoglobulins are the most abundant proteins in dromedary colostral whey (101.8 g/L), but the concentration rapidly decreases to 19.6 g/L at 48 hours after birth and reaches 7.9 g/L at 192 hours after birth (El-Hatmi *et al*., 2007). Zhang *et al*. (2005) also found a similar trend for Alxa Bactrian camel. α-Lactalbumin and blood serum albumin are the dominant whey proteins of mature milk in Bactrian camel (Zhang *et al*., 2005) and dromedary camel (El-Hatmi *et al*., 2006; El-Agamy *et al*., 2009) using densitometry of SDS-PAGE gel images. By contrast, α-lactalbumin and lactoferrin are the major proteins in human mature milk whey (El-Agamy *et al*., 2009). A comparison of whey protein band patterns of several farm animal milks is shown in Fig. 26.4.

The catalytic efficiency of chymotrypsin is the same for bovine and camel whey proteins, while that of trypsin for bovine whey is slightly higher than that for camel whey (Salami *et al*., 2008). However, camel α-lactalbumin shows significantly higher digestibility by pancreatic proteases and antioxidant activity than bovine α-lactalbumin, suggesting a potential benefit of camel α-lactalbumin as content in infant formula (Salami *et al*., 2009). Bactrian and dromedary camel milk whey possess three and two α-lactalbumin variants, respectively (Ochirkhuyag *et al*., 1998).

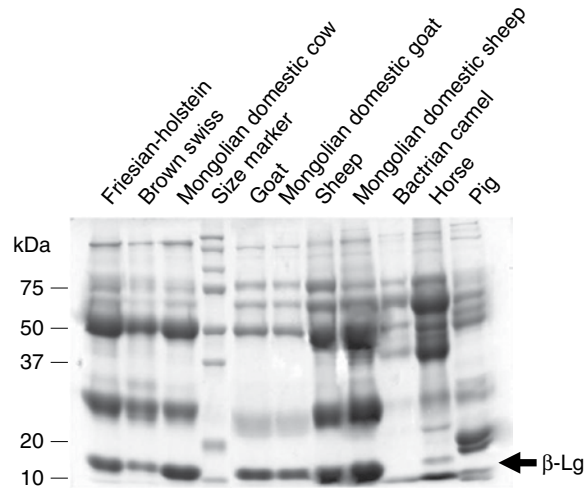


Figure 26.4. Coomassie Brilliant Blue R-250 staining of SDS-PAGE of whey fractions prepared from several farm animal milks. Bands corresponding to β-lactoglobulin are indicated by the arrow.

A prominent characteristic of Bactrian and dromedary camel milks, when compared with cow milk, is the absence of β-lactoglobulin, which is the main component (50%) of cow whey proteins (Ochirkhuyag *et al*., 1997; Merin *et al*., 2001; Kappeler *et al*., 2003; Zhang *et al*., 2005; El-Hatmi *et al*., 2007). Human milk lacks β-lactoglobulin but its presence in cow milk causes allergenicity in humans; thus its absence in camel milk can be seen as an advantage over cow milk (El-Agamy *et al*., 2009).

Lactoferrin, a member of the transferrin family, is an 80-kDa glycoprotein that is capable of chelating two ferric ions. It shows a wide variety of defensive effects, such as anti-inflammatory, antimicrobial, antitumor, and immunomodulatory activities. The concentration of lactoferrin in normal dromedary camel milk ranges from 0.02 to 2.1 g/L (Al-Majali *et al*., 2007). Konuspayeva *et al*. (2007) reported that Bactrian camels in Kazakhstan had a milk lactoferrin concentration of 0.223 ± 0.132 g/L, similar to the range found in dromedary milk (0.209 ± 0.131 g/L). Camels in Kazakhstan showed little variation in lactoferrin content between different regions and breeds, but a significant seasonal variation has been observed (Konuspayeva *et al*., 2007). In contrast, Ereifej *et al*. (2011) observed wide variation in lactoferrin content, from non-detectable to 0.43% of total milk protein, while El-Hatmi *et al*. (2007) reported variation in temporal individual production of lactoferrin, from non-detectable to a maximum of 2.3 g/L at 48 hours after birth; this may reflect the health of the milked camels. Camel lactoferrin showed the highest bactericidal activity against enterohemorrhagic *Escherichia coli* O157, H7 when compared with sheep, goat, alpaca, elephant, and human lactoferrin (Conesa *et al*., 2008). Lysozyme is another antimicrobial agent in milk; its concentration in dromedary camel milk (150 μg/L) has been reported to be higher than that in cow milk (70 μg/L) (El-Agamy *et al*., 1998).

Immunoglobulins in milk play a significant role in passive immunity of neonates, especially during the initial stage of lactation. IgG is the dominant immunoglobulin in camel milk. It is secreted at a concentration of around 100 g/L in colostrum but decreases rapidly to around 20 g/L within 2 days followed by a gradual decline to less than 10 g/L (El-Hatmi *et al*., 2006, 2007; Konuspayeva *et al*., 2007). Among the three immunoglobulin variants (IgG1, IgG2, and IgG3), homodimeric IgG2 and IgG3 in Camelidae serum were found to comprise a functional heavy chain, consisting of a single variable domain and two constant domains, but no light chains (Hamers-Casterman *et al*., 1993). Interestingly, the recombinant variable domain, termed "nanobody," was produced without any aggregation, and it maintained binding capacity against antigens at an equivalent level as the original heavy-chain antibodies (Arbabi Ghahroudi *et al*., 1997). The major portion of the IgG variants in milk arises from the serum (Marnila & Korhonen, 2011). The heavy-chain antibodies exist in camel milk, as El-Hatmi *et al*. (2007) reported that IgG2 and IgG3 were secreted at 58.4 and 5.7 g/L at 1 and 192 hours after birth, respectively.

26.4 MILK ALLERGY

Infants who are allergic to the proteins in cow milk, such as caseins and β-lactoglobulin, suffer a severe immune response when they ingest non-human milk. The symptoms can be fulminant and sometimes even lethal, and hence many studies have been done to reduce the allergenicity of non-human milks or to find milks that can be substituted for cow milk without producing an allergenic response (El-Agamy, 2006). The former approach has been successful in terms of reducing allergenicity, but frequently it results in a product with reduced nutritional value. Therefore, appropriate hypoallergenic substitute milks are needed.

Camel milk is likely to be beneficial for infants allergic to cow milk because it lacks β-lactoglobulin, as does human milk, and because the high ratio of whey proteins to casein in camel milk makes a soft and easily digestible curd (El-Agamy, 2006). It is also evident from *in vitro* and *in vivo* experiments that camel milk is hypoallergenic and a promising substitute for children who are allergic to cow milk (Restani *et al*., 1999; El-Agamy *et al*., 2009; Ehlayel *et al*., 2011). However, a case, maybe a rare instance, of anaphylaxis to camel milk in an atopic child has been

reported (Al-Hammadi *et al*., 2010). An acute milk allergy is usually obvious and easy to diagnose by skin-prick test, but a chronic milk allergy is not and therefore careful clinical validation (both *in vitro* and *in vivo*) for drinking camel milk is required, especially for those children at risk of developing serious allergic reactions (Al-Hammadi *et al*., 2010).

26.5 HEALTH-BENEFICIAL MICROORGANISMS IN CAMEL MILK AND ITS PRODUCTS

26.5.1 Lactic acid bacteria

Lactic acid bacteria (LAB) are the key microorganisms in terms of dairy food preservation and production of fermented dairy products. Furthermore, LAB that can reach and colonize the human intestine are called probiotics and these maintain the natural gastrointestinal microbiota and exhibit health-beneficial effects to the host. Several species of *Enterococcus*, *Pediococcus*, *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Weissella* have been found in raw camel milk and its dairy products (Table 26.5). In general, lowering the pH of dairy foods by lactic acid (Browning, 1886) produced by LAB and antimicrobial agents such as bacteriocin derived from LAB (Klaenhammer, 1988) can halt the growth of pathogenic microorganisms. Milk proteins are digested by the action of proteases derived from LAB during the fermentation process, and this leads to improvement in the digestibility of milk proteins and promotes their efficient intake in the small intestine of humans. Moreover, a variety of peptides encrypted in milk proteins are also known to be released during the fermentation process and exhibit several health-beneficial activities such as antibacterial (Benkerroum, 2010), antihypertensive (Yamamoto, 1997; Saito, 2008), antioxidant (Kudoh *et al*., 2001), antitumor (LeBlanc *et al*., 2002), and immunomodulating (Gill *et al*., 2000; LeBlanc *et al*., 2002).

A nonapeptide derived from κ-casein, Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp (κ-casein f107–115), has been produced by fermentation of bovine skim milk with *Lactobacillus helveticus* 130B4 isolated from fermented milk of Chinese Bactrian camels (Shuangquan *et al*., 2008). This peptide showed *in vitro* inhibitory activity against angiotensin I-converting enzyme, a dipeptidyl carboxypeptidase associated with raised blood pressure (IC_{50} 19.9 μmol/L). The IC_{50} of the nonapeptide was comparable to that found for cow milk-derived antihypertensive peptides (IC_{50} values ranging from 2 to 1682 μmol/L) (Saito, 2008). The internal sequence of the nonapeptide, Ile-Pro-Pro, is known as a β-casokinin or a lactotripeptide (IC_{50} 5 μmol/L), and is released during the fermentation of cow milk with *Lactobacillus helveticus* and *Saccharomyces cerevisiae* (Nakamura *et al*., 1995).

Potential probiotic LAB strains have been isolated from traditional dairy products made from camel milk. *Shmen*, an Algerian traditional butter made from dromedary camel milk, yielded two strains of *Lactobacillus plantarum*, SH12 and SH24, which have been found to be tolerant to low pH and 2% bile (Kacem & Meriem, 2008). These two strains likely produced bacteriocins that showed inhibitory activity against cell proliferation of *Lactococcus lactis* B8. From several traditional Mongolian dairy products, Takeda *et al*. (2011) selected 115 LAB strains that could survive low pH and 0.2% bile. Of the 115 strains, 10 (five *Lactobacillus plantarum*, one *Lactobacillus delbrueckii* subsp. *lactis*, two *Lactobacillus paracasei* subsp. *tolerans*, and two *Lactobacillus paracasei* subsp. *paracasei*) were capable of adhering to the surface of Caco-2 cells, an intestinal cell line derived from a human colorectal carcinoma that spontaneously differentiates under standard culture conditions. Among the 10 potential probiotics in the traditional Mongolian dairy products, six (two *Lactobacillus plantarum*, two *Lactobacillus paracasei* subsp. *tolerans*, and two *Lactobacillus paracasei* subsp. *paracasei*) were isolated from *tarag*, a yogurt made from Bactrian camel milk.

There is a question from the hygiene point of view on consecutive inoculation of *gariss* as starter culture, because the presence of a potentially pathogenic *Streptococcus infantarius* subsp. *infantarius*, which contains a gene encoding a virulence determinant *gtf*, has been confirmed (Abdelgadir *et al*., 2008). This indicates the importance of precise analysis and regular monitoring of microflora in traditional dairy products.

26.5.2 Yeasts

Some yeasts capable of metabolizing lactose or lactate can be present in mammalian milk. Together with LAB, such yeasts contribute substantially to the fermentation process of dairy products. For example, ethanol is produced by a spontaneous yeast/lactic fermentation, and yeasts also synthesize a wide range of secondary metabolites such as aldehydes, esters, ketones, and organic acids that occasionally confer favorable flavor to the fermented products.

More than 10 yeast strains have been found in traditional dairy products made from camel milk (Table 26.6), including *gariss* (Sulieman *et al*., 2006; Abdelgadir *et al*., 2008), *hogormag* (Shuangquan *et al*., 2004), *shmen* (Kacem & Karam, 2006), *shubat* (Rahman *et al*., 2009), *suusac* (Lore *et al*., 2005), and *tarag* (Watanabe *et al*., 2008). *Gariss* contains 1.4 ± 0.03% ethanol, indicating the occurrence of yeast/lactic fermentation (Sulieman *et al*., 2006). Ripening of *shmen*, Algerian traditional butter made from dromedary camel milk, may be affected by the strong lipolytic activity of yeasts (Kacem & Karam, 2006).

Table 26.5. Lactic acid bacteria isolated from raw camel milk and its fermented milk products.

Sources and species	Reference
Algerian dromedary camel milk *E. durans*, *E. faecalis*, *E. faecium*, *Lb. paracasei* subsp. *paracasei*, *Lb. plantarum*, *Lb. rhamnosus*, *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *lactis* biovar *diacetylactis*	Hassaïne *et al*. (2007), Drici *et al*. (2010)
Moroccan dromedary camel milk *E. casseliflavus*, *E. faecalis*, *E. faecium*, *P. acidilactici*, *P. damnosus*, *P. halophilus*, *P. paravulus*, *P. pentosaceus*, *Strep. bovis*, *Strep. salivarius*, *Strep. salivarius* subsp. *thermophilus*, *Lb. amylophilus*, *Lb. brevis*, *Lb. case*i subsp. *casei*, *Lb. casei* subsp. *rhamnosus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Lb. paracasei* subsp. *tolerans*, *Lb. plantarum*, *Lc. garviae*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, *Lc. raffinolactis*, *Leu. lactis*, *Leu. mesenteroides* subsp. *cremoris*, *Leu. mesenteroides* subsp. *dextranicum*, *Leu. mesenteroides* subsp. *mesenteroides*	Benkerroum *et al*. (2003), Khedid *et al*. (2009)
Cheese made from Indian dromedary camel milk *Lb. casei*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. fermentum*, *Lb. plantarum*	Nanda *et al*. (2011)
Gariss (fermented Sudanese dromedary camel milk) *E. faecium*, *Strep. bovis*, *Strep. infantarius* subsp. *infantarius*, *Lb. animalis*, *Lb. brevis*, *Lb. divergens*, *Lb. fermentum*, *Lb. gasseri*, *Lb. helveticus*, *Lb. paracasei* subsp. *paracasei*, *Lb. plantarum*, *Lb. rhamnosus*, *Lactobacillus* sp., *Lc. alimentarium*, *Lc. lactis*, *Lc. raffinolactis*	Sulieman *et al*. (2006), Abdelgadir *et al*. (2008), Ashmaig *et al*. (2009)
Hogormag (fermented Chinese Bactrian camel milk) *E. faecium*, *Lb. acidophilus*, *Lb. bavaricus*, *Lb. casei*, *Lb. helveticus*, *Lb. plantarum*, *Lc. lactis* subsp. *cremoris*, *Leu. lactis*	Shuangquan *et al*. (2004)
Shmen (Algerian traditional butter made from dromedary camel milk) *E. faecium*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. plantarum*, *Lb. paracasei* subsp. *paracasei*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, *Leu. gelidum*, *Leu. pseudomesenteroides*	Kacem & Karam (2006)
Shubat (fermented Chinese Bactrian camel milk) *E. faecalis*, *E. faecium*, *Lb. brevis*, *Lb. helveticus*, *Lb. sakei*, *Leu. lactis*, *W. helleca*	Rahman *et al*. (2009)
Suusac (fermented Kenyan dromedary camel milk) *Lb. curvatus*, *Lb. plantarum*, *Lb. salivarius*, *Lc. raffinolactis*, *Leu. mesenteroides* subsp. *mesenteroides*	Lore *et al*. (2005)
Tarag (yogurt made by Mongolian Bactrian camel milk) *E. faecium*, *P. paravulus*, *Lb. casei*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. fermentum*, *Lb. helveticus*, *Lb. kefiranofaciens*, *Lb. kefiri*, *Lb. paracasei* subsp. *paracasei*, *Lb. paracasei* subsp. *tolerans*, *Lb. pentosus*, *Lb. plantarum*, *Lc. lactis* subsp. *lactis*, *Leu. citreum*, *Leu. mesenteroides*	Watanabe *et al*. (2008), Takeda *et al*. (2011)

E, *Enterococcus*; *Lb*, *Lactobacillus*; *Lc*, *Lactococcus*; *Leu*, *Leuconostoc*; *P*, *Pediococcus*; *Strep*, *Streptococcus*; *W*, *Weissella*.

Table 26.6. Yeasts isolated from traditional fermented camel milk products.

Sources and species	Reference
Gariss (fermented Sudanese dromedary camel milk) *I. orientalis*, *Kl. marxianus*	Sulieman *et al*. (2006), Abdelgadir *et al*. (2008)
Hogormag (fermented Chinese Bactrian camel milk) *C. glabrata*, *C. kefyr*, *C. krusei*, *S. cerevisiae*	Shuangquan *et al*. (2004)
Shmen (Algerian traditional butter made from dromedary camel milk) *S. cerevisiae*, *Saccharomyces* sp.	Kacem & Karam (2006)
Shubat (fermented Chinese Bactrian camel milk) *C. ethanolica*, *K. unispora*, *Kl. marxianus*	Rahman *et al*. (2009)
Suusac (fermented Kenyan dromedary camel milk) *C. krusei*, *G. penicillatum*, *R. mucilaginosa*	Lore *et al*. (2005)
Tarag (yogurt made by Mongolian Bactrian camel milk) *I. orientalis*, *K. unispora*, *P. mandshurica*, *S. cerevisiae*, *T. delbrueckii*	Watanabe *et al*. (2008)

C, *Candida*; *G*, *Geotrichum*; *I*, *Issatchenkia*; *K*, *Kazachstania*; *Kl*, *Kluyveromyces*; *P*, *Pichia*; *R*, *Rhodotorula*; *S*, *Saccharomyces*; *T*, *Torulaspora*.

Symbiotic relationships can be considered between LAB and yeasts in these traditional dairy products; however, the contribution of yeasts to the fermentation process of camel milk is not fully understood and hence further investigations will be required.

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27
Horse and Donkey Milk

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27.1 INTRODUCTION

The horse (*Equus caballus*) and the donkey (*Equus asinus*) are 'historical' dairy species belonging to the order Perissodactyla, the family Equidae and the genus *Equus*. Their domestication about 5000 years ago was mainly for draught and transport purposes and had a great impact on the socioeconomic development of humanity (Beja-Pereira *et al*., 2004) in terms of communication and, unfortunately, warfare. The health-promoting properties of milk from these equids were greatly appreciated in ancient times as documented by Hippocrates and Pliny the Elder. Donkey milk is also used for therapeutic purposes in some African societies (Pearson *et al*., 2005) and fermented horse milk (*koumiss* or *airag*) with claimed functional and therapeutic properties is traditionally produced and consumed in Eurasian steppe regions right up to Central Europe. In this regard, official data on Mongolia horse milk production, mainly used for *koumiss* production, report an increase from 43 300 t in 2006 (Tsetsgee & Ugdill, 2007) to 100 000 t in 2011 (Tsetsgee & Damdinsuren, 2012).

27.2 WORLDWIDE HORSE AND DONKEY DISTRIBUTION AND MILK PRODUCTION

To the best of our knowledge no other official data are available on global horse or donkey milk production. These equids are differently distributed among countries (Table 27.1) and this is related to their evolution in different environmental conditions. Equines contribute significantly to both rural economies and quality of life around the world: horses are more represented in cold cool areas while donkeys, a source of animal power, are mainly present in arid and semi-arid areas. In summary, Food and Agriculture Organization (FAO) data report, as live animals, more than 59 million horses in the world (2009 data; FAOSTAT, 2011), of which the American continent has 56%, Asia 24%, Europe 11%, Africa 8% and Oceania 1%.

The available published data on horse milk production is also negligible and very variable, ranging from the previously mentioned 100 million litres in Mongolia to 1 million litres in Europe (Fox & Uniacke, 2010). According to Langlois (2010), production of horse milk dates back to the Botai society (Kazakhstan, 3500 BC) and is still a distinctive animal production system in Kazakhstan, Kyrgyzstan, the Russian Federation, Uzbekistan, Mongolia, Tibet and Xinjiang, China. In the extensive systems of Eurasian areas, crossbreed mares are usually milked in Mongolia but it is reported that autochthonous breeds have been selected for this productive trait, as is the case for dairy breeds: Jade Kazakh, Draft Kazakh, Kushum, Russian draft horse, Lithuanian draft horse, Lokai and Novokirghiz (Doreau & Martin-Rosset, 2011). Many factors can profoundly affect horse milk yield, estimated to be between 30 and 400 L per lactation in extensive systems (Langlois, 2010) or 1000–2500 L per lactation in European equine dairy farms (France, Germany, the Netherlands, Hungary, Bulgaria, Belarus, Ukraine and Italy) (Doreau & Martin-Rosset, 2011; G.F.W. Haenlein, personal communication).

With the evolution of more industrialised societies, animal power is being increasingly replaced by mechanisation

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Table 27.1. Worldwide distribution (millions) of horses and donkeys.

Continent	Country	Horses	Donkeys
Asia		13.985	17.798
	Afghanistan	0.177	1.322
	China	6.731	6.823
	India	0.751	0.650
	Iran	0.140	1.600
	Kazakhstan	1.370	0.030
	Mongolia	2.221	0.228
	Pakistan	0.350	4.509
	Russian Federation	1.353	0.019
Africa		4.885	18.973
	Burkina Faso	0.036	1.000
	Botswana	0.038	0.330
	Chad	0.409	0.405
	Ethiopia	1.995	5.715
	Egypt	0.067	3.350
	Mali	0.111	1.767
	Morocco	0.153	0.962
	Namibia	0.045	0.140
	Niger	0.239	1.598
	Nigeria	0.207	1.050
	Senegal	0.522	0.441
	Sudan	0.026	0.751
America		33.421	7.015
	Argentina	3.680	0.098
	Brazil	5.496	3.260
	Bolivia	0.475	0.635
	Colombia	2.505	1.030
	Haiti	0.210	0.630
	Mexico	6.350	3.260
	Peru	0.730	0.630
	USA	9.500	0.052
	Venezuela	0.510	0.440
Europe		6.362	0.633
	Albania	0.041	0.064
	Bulgaria	0.175	0.130
	France	0.460	0.015
	Germany	0.545	
	Greece	0.027	0.040
	Ireland	0.098	0.006
	Italy	0.300	0.024
	The Netherlands	0.133	
	Poland	0.297	
	Portugal	0.019	0.125
	Romania	0.820	0.030
	Spain	0.250	0.142
	Ukraine	0.465	0.012
	UK	0.384	

Source: based on 2009 data from FAOSTAT (2011).

and there is global concern about the permanent loss of draft-horse breeds and most donkey breeds (Miraglia *et al*., 2003; Chen *et al*., 2010). For this reason, both saddle horse breeds (e.g. Haflinger, Anglo-Arabian) and endangered draft horse breeds (e.g. Breton, Comtois, Franches Montagnes, Bardigiano) are reported to be used for milk production, in a system involving multipurpose working animals (Salimei & Fantuz, 2012) also including mule production (Miraglia *et al*., 2003). Moreover, a dairy donkey farming system has been recently developed in Europe (Salimei, 2011) where donkey farms are mainly located in marginal areas of Italy, France, Portugal, Spain, Greece and Belgium. As for horse, donkey milk production is frequently an activity within multipurpose farming systems, including agro and equestrian tourism, educational and social activities, pet therapy, organic health farms, beauty farms, even street vendors (e.g. in Santiago, Chile and in Bolivia). There is ample variability reported in the literature for donkey milk production, which varies from 500 L per 300-day lactation (Giosuè *et al*., 2008) to 235 L per 180-day lactation (Guo *et al*., 2007). The data on donkey milk production are mainly from Martina Franca, Ragusana, Grigio Siciliano, Jiangyue and Littoral-Dinaric donkey breeds, but crossbred jennies are also intensively milked on dairy donkey farms.

The above population numbers gain meaning in terms of potential equine dairying if we consider that (i) the largest horse population (9.5 million) is located in the USA, which only has 52 000 donkeys; and (ii) that the world donkey population is estimated to be 43.5 million (FAOSTAT, 2011). China has the largest donkey population (6.8 million), but Africa has the largest percentage (44%), followed by Asia (39%), Latin America (16%) and Europe (1%). Donkeys are mainly present in areas characterised by stagnating offers of agricultural products (Fall *et al*., 2003) and high incidence of malnutrition in children and adults. Thanks to their composition, both horse and donkey milk can therefore be considered an under-valued animal protein source in the human diet for both developing and industrialised countries.

In addition to the data from the FAO reported in Table 27.1, another source of data has estimated a donkey population in the UK (2007) of 8954 animals, the majority of which are owned by The Donkey Sanctuary, a worldwide animal charity that fosters abused donkeys and mules (Cox *et al*., 2010) and these animals are involved in therapy for children with special needs (Svendsen, 1997).

27.2.1 Horse and donkey milk production for human consumption

In this section some details will be given to elucidate the essentials of the dairy equid management system, which is clearly influenced by anatomical and physiological

differences compared with conventional dairy species. The most important factors are as follows:

1. The equid mammary gland has a small volume (maximum 2–2.5 L) with limited cisternal capacity.
2. There is a large proportion of alveolar milk (75–85%).
3. The residual milk, averaging 30% of total milk, has the highest fat content.
4. For satisfactory persistence of lactation, dams and foals are stabled together until natural weaning (6–9 months old) and are separated 3 hours before each daily milking. An interval between mammary evacuations (by foals or milking) of longer than 3 hours is not recommended because, in general, reducing the frequency of mammary gland evacuation (milk stasis) decreases milk secretion rate, particularly in species with a low proportion of cisternal milk (Silanikove *et al*., 2010) as is the case for equids. In commercial horse milk farms, mares can be milked, depending on market demand, three to five times per day to provide as much as 15 kg daily milk yield, while foals are separated from the mares for longer periods.
5. The presence of the foal during milking is required for most horses in order to complete milk extraction; training and selective breeding of mares without this requirement should be considered.
6. The wavering market demand for horse and donkey milk is due to sparse availability and lack of communication (Salimei & Fantuz, 2012), but the internet, new food technologies and commercial shipping of milk, fermented or not, could help wider distribution.

As a consequence:

1. raw milk is often produced 'on request' although larger farms also produce freeze-dried horse and donkey milk; and
2. dams could be milked up to eight times a day in specific milking facilities or stables following strict procedures for preparation and management of milking (Simoni *et al*., 2004; Salimei & Fantuz, 2012), but no more than two to three milkings should be the routine.

Another important difference from the conventional ruminant dairy species is that horse and donkey nutrition has a direct influence on the nutritional and sensorial qualities of the milk of these monogastric species.

Mechanical milking should be preferred in the dairy equid farming system, as it improves udder evacuation, as reported for Murgese horses (Caroprese *et al*., 2007), and reduces the risk of microbial contamination of milk (Colavita *et al*., 2011) compared with hand milking. Cleaning and sanitation of milking equipment and the hygiene of dairymen are crucial elements when raw milk is commercialised so that restrictive rules are applied to milk production at local levels (see section 27.9.1) (Colavita *et al*., 2011).

Sheep milking machines have been adapted to equid milking and they are often set at 120 cycles/min, 50% pulsation rate and vacuum levels lower than 50 kPa (Doreau & Martin-Rosset, 2011; Salimei, 2011). The equid milk somatic cell count is reported to range between 3200 and 355 000 cells/mL (Pecka *et al*., 2012; Salimei & Fantuz, 2012). Mastitis is rare in equids but management of lactating animals could have a negative impact on the health status of the mammary gland in intensive farming systems.

With regard to animal welfare, dams are not milked during the first 30–45 days of lactation and concurrent lactation and pregnancy in the horse do not generate a mother–offspring conflict during intensive lactation (Bartosova *et al*., 2011). The milking system does not influence the behavioural activities of horses (Caroprese *et al*., 2007). Studies have shown that donkeys adapt quickly to milking management and facilities (Salimei, 2011).

Methodological differences among studies on horse and donkey milk yield result in a high variability of milk production in both species (Coenen *et al*., 2011; Salimei & Fantuz, 2012). However, consistent with the average growth rate of neonatal horses (foals double their weight within 42 days), lactation likely peaks within the second month of lactation in horses (Coenen *et al*., 2011). Modelling milk yield of a light-horse breed (Lusitano, 400–500 kg body weight) using Wood's equation showed the lactation peak on day 31 after foaling (14 kg milk per day) with a monthly persistence of 90–95% (Santos & Silvestre, 2008). Available data on milk production for different horse breeds are inconclusive, so milk yield (see section 27.2) is frequently provided as a percentage of the dam's body weight and ranges from 30 to 33.5 g/kg body weight per day (Doreau & Martuzzi, 2006; Coenen *et al*., 2011). Data on donkey milk production, less abundant and more recent than those on horse milk, are mainly reported in the literature as millilitres of milk per milking session, including 3 hours separation from foals and milking routine. Donkey milk yield is highly variable because of different experimental conditions, as shown in Fig. 27.1 for manual and mechanical milking. Ample individual variation is reported in the literature for donkey milk production (Salimei & Fantuz, 2012) so that breeding selection could improve the dairy traits of the species, as already described for horses.

27.3 GROSS COMPOSITION AND PHYSICAL PROPERTIES OF HORSE AND DONKEY MILK

When compared with milk from different species equid milk shows closer resemblance to human milk, with a relatively low protein and ash and a high lactose content

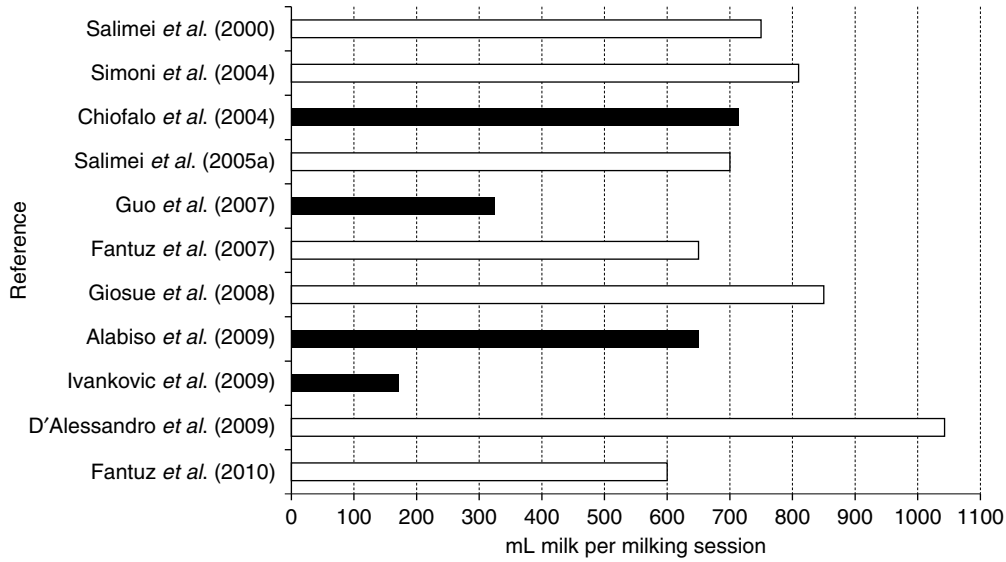


Figure 27.1. Donkey milk yield under different experimental conditions. Open bars indicate mechanical milking; filled bars indicate manual milking. Based on data from the reference sources shown in the diagram. Also based on data from Salimei & Fantuz (2012).

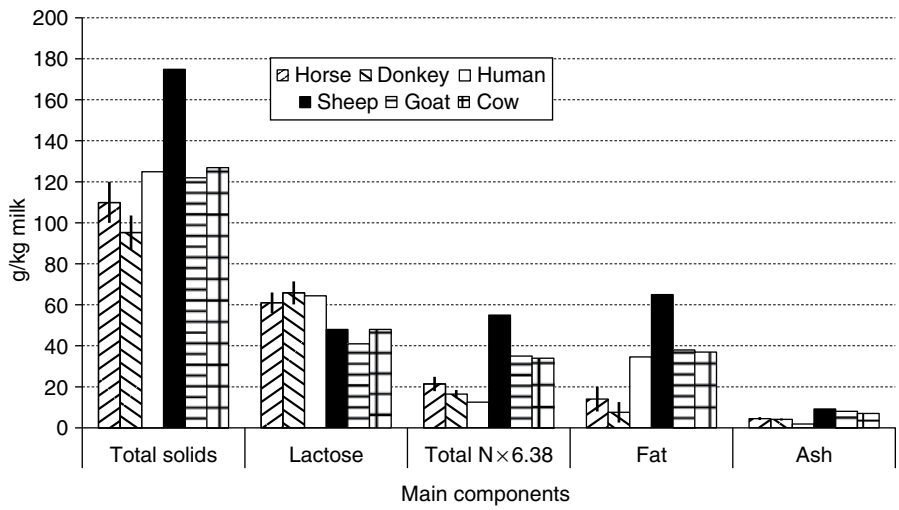


Figure 27.2. Gross composition of donkey and horse milk (mean ± SD) compared with human, cow, sheep and goat milk. Adapted from Salimei (2011), with permission of Academic Press. Modified using further data from Park *et al.* (2007) and Doreau & Martin-Rosset (2011).

(Fig. 27.2). Equid milk fat content (Fig. 27.2) is remarkably low, which results in a low energy content: 2.10 and 1.76 MJ/kg for horse and donkey milk, respectively (Salimei & Fantuz, 2012). In horse milk, the fat accounts for only 10–20% of gross energy (Mariani *et al.*, 2001), whereas the percentage of energy from fat in human milk ranges from 45 to 55% (Milligan, 2010). Fat is the most variable component in milk from dairy donkeys and its content is reported to be even lower than in horse milk. The fat content in donkey milk is related to the efficiency of milk removal from the mammary gland. Residual milk, obtained by exogenous oxytocin injection following

Table 27.2. Changes in the composition of mammary secretion (g/kg mammary secretion) within 96 hours after foaling in horse and donkey.

		0	6 h	12 h	24 h	36 h	48 h	72 h	96 h
Total solids	Horse	227	124	119	117		119	118	113
	Donkey		192		137	116	119		
Fat	Horse	7.1	25	27	25		25	25	19
	Donkey		23		35	27	20		
Protein	Horse	175	49	36	33		32	31	31
	Donkey		117		33	32	35		

Source: based on data from Salimei & Fantuz (2012).

Table 27.3. Physical properties of horse, donkey, human and cow milk.

	Horse	Donkey	Human	Cow
pH	7.1–7.3	7.01–7.35	7.0–7.5	6.5–6.7
Density (kg/m^3)	1033–1035	1029–1037	1031	1027–1033
Freezing point (°C)	–0.554 to –0.548	–0.540 to –0.508		–0.550 to –0.512
Viscosity (mPa s)	1.503			1.631

Sources: based on data from Guo *et al*. (2007), Salimei (2011) and Uniacke-Lowe & Fox (2011).

normal machine milking, contains higher milk fat content (1.73%) than milk previously released by normal milking (0.43%) (Simoni *et al*., 2004). Variation in the fat content of donkey milk is also associated with milking strategies: a higher fat content was found when there were longer intervals between milking sessions (Salimei, 2011).

Mammary secretion changes with the advance of lactation: equid colostrum contains a higher protein content than mature milk, mostly represented by immunoglobulins and enzymes (Uniacke-Lowe *et al*., 2010). Compared with cows, the colostral period is shorter in equids with the main components reaching levels close to mature milk within 24–36 hours after parturition (Table 27.2). According to Salimei and Fantuz (2012), after the colostral period the lactose and fat contents remain substantially stable in the milk but decreasing trends for fat and increasing trends for lactose have been observed during advancing lactation. The protein content decreases by approximately 20–25% in horse and donkey milk from 28 to 150 days after foaling, mostly due to the casein content, which during the same period decreases by 20–30% in the milk from both species. The ash content also decreases in mature equid milk with advancing lactation.

The gross composition of donkey milk is affected by the circadian rhythm, with higher fat and lactose contents at night and a higher protein content during the day (Piccione *et al*., 2008). In horse milk the percentage of fat is influenced by nutritional factors such as body condition score and dietary energy to protein ratio (Doreau & Martuzzi, 2006; Martuzzi & Doreau, 2006). Dietary trace element supplementation to lactating donkeys or the substitution of wheat bran (20% inclusion) in the concentrate (2.5 kg/day) with dehydrated beet pulp (20% inclusion) does not affect daily milk yield or milk gross composition (Salimei *et al*., 2005a; Fantuz *et al*., 2010). Differences between breeds have also been reported for the nitrogen fraction and ash content of horse milk but results are not conclusive (Martuzzi & Doreau, 2006; Uniacke-Lowe *et al*., 2010).

As far as the physical properties of the milk are concerned (Table 27.3), similarly to human, horse and donkey milk have a sub-alkaline pH compared with the sub-acidic pH of cow milk. This is probably due to differences in casein and salt composition. Horse milk has a lower freezing point and is less viscous compared with cow milk, in relation to the higher lactose content and to the lower total solids (Uniacke-Lowe & Fox, 2011).

27.4 NITROGEN FRACTION OF HORSE AND DONKEY MILK

Milk nitrogen content can be broadly divided into two groups: true protein and non-protein nitrogen. True protein includes two main classes: caseins and whey proteins. Caseins and some whey proteins such as α-lactalbumin (ALA) and β-lactoglobulin (BLG) are synthesised by the mammary gland, whereas other whey proteins such as

Table 27.4. Distribution of nitrogen fraction in horse, donkey, human and cow milk (data are expressed per 100 g of milk) and relative percentage of total nitrogen (in parentheses).

	Horse	Donkey	Human	Cow
Total nitrogen (mg)	335.4 (100)	258.7 (100)	222.6 (100)	509.4 (100)
Casein nitrogen (mg)	167.7 (50.0)	118.3 (45.7)	58.0 (26.0)	393.4 (77.2)
Whey nitrogen (mg)	130.1 (38.8)	107.5 (41.5)	119.1 (53.5)	89.3 (17.6)
Non-protein nitrogen (mg)	37.6 (11.2)	36.1 (14.0)	45.4 (20.4)	26.6 (5.2)
Crude protein (g)	2.14	1.65	1.42	3.25
True protein (g)	1.90	1.42	1.11	3.08
Caseins (g)	1.07	0.75	0.37	2.51
Whey proteins (g)	0.83	0.68	0.76	0.57

Sources: based on data from Malacarne *et al*. (2002) and Fantuz *et al*. (2009a).

Table 27.5. Distribution (% of total caseins) of individual caseins in horse, donkey, human and cow milk.

	Horse	Donkey	Human	Cow
Caseins (g/100 g)	1.10	0.76	0.48	2.60
α_{s1}-Casein (%)	17.9	Identified	32	41
α_{s2}-Casein (%)	1.4	Identified	Not identified	10.8
β-Casein (%)	78.5	Identified	Max. 85	33
κ-Casein (%)	1.8	Identified	<15	12

Sources: based on data from Uniacke-Lowe *et al*. (2010) and Salimei & Fantuz (2012).

serum albumin and immunoglobulins are derived from the blood (Emmet & Rogers, 1997).

Casein nitrogen in donkey and horse milk is much lower than in ruminant milk, and the casein proportion represents only 46 and 50% of milk total nitrogen, whereas whey nitrogen represents 41 and 39% of total nitrogen in donkey and horse milk, respectively. The whey protein fraction represents 53% of total protein in human milk and 18% in cow milk, the casein proportion being 26% and 77%, for human and cow milk, respectively (Table 27.4). The main proteins identified in horse and donkey milk are α_{s1}-, α_{s2}-, β- and κ-casein, BLG, ALA, serum albumin, lysozyme, lactoferrin and immunoglobulins (Salimei *et al*., 2004; Vincenzetti *et al*., 2008; Chianese *et al*., 2010; Uniacke-Lowe *et al*., 2010; Cunsolo *et al*., 2011).

27.4.1 Caseins

The main biological function of caseins is to provide amino acids, to transport in liquid form a large amount of calcium and phosphorus, and to provide bioactive peptides to the newborn (Fox & Brodkorb, 2008; Uniacke-Lowe *et al*., 2010). Compared with horses, data on donkey caseins are scarce and individual caseins have not yet been quantified. Individual caseins occur in different proportions in milk from different species (Table 27.5): β-casein is the most represented in horse, goat and human milk whereas in cow milk α_{s1}-casein has the highest proportion (Uniacke-Lowe *et al*., 2010). Furthermore, compared with cows, horse milk contains a very low level of κ-casein (Table 27.5). The presence of γ-casein in horse and donkey milk (Egito *et al*., 2002; Salimei *et al*., 2004) is mostly related to the hydrolysis of β-casein by the action of milk endogenous proteases such as plasmin, whose activity is reported to be higher in horse milk than in cow milk (Humbert *et al*., 2005).

The molecular masses of horse α_{s1}-casein (205 amino acid residues), β-casein (226 amino acid residues) and κ-casein (165 amino acid residues), prior to post-translational modification, are 26 614.4, 25 511.4 and 18 844.7 Da, respectively, and two smaller isoforms of horse α_{s1}- and β-casein have also been reported (Uniacke-Lowe *et al*., 2010). Horse caseins contain a low level of α_{s2}-casein (Egito *et al*., 2002), of which the complete amino acid sequence has not yet been determined, and identification of the 12–15 amino acids of the N-terminal region is not conclusive (Uniacke-Lowe & Fox, 2011). At present there is no evidence of the presence of α_{s2}-casein in human milk.

Mateos *et al*. (2009) have identified 36 variants of equine α_{s1}-casein, with the number of phosphate groups (P) ranging from two to six or eight. The β-casein isolated from Haflinger horse milk is composed of highly multiphosphorylated isoforms with three to seven phosphate groups, which may explain the micro-heterogeneity shown by urea and two-dimensional electrophoresis (Girardet *et al*., 2006; Mateos *et al*., 2010). Human β-casein contains between

zero and five phosphate groups per molecule and the bovine counterpart, which is reported to be fully phosphorylated on its five potential sites, contains a mean ratio of 4.9P (Uniacke-Lowe *et al*., 2010). In horse milk, κ-casein is reported to be more glycosylated than its bovine counterpart (Martuzzi & Doreau, 2006).

In donkey milk the presence of all four individual caseins has only recently been reported (Chianese *et al*., 2010; Cunsolo *et al*., 2011). The compositional heterogeneity shown by donkey α_{s1}-casein is attributed to phosphorylation (5, 6 or 7P) and to the presence of non-allelic deleted forms generated by RNA splicing (Chianese *et al*., 2010). In donkey milk (51 samples) from the Ragusana breed, one case of milk protein profile lacking two components of α_{s1}-casein has been reported (Criscione *et al*., 2009). Donkey α_{s2}-casein contains 10, 11 or 12P whereas β-casein contains 5, 6 or 7P. The presence of a novel β-casein with a higher molecular weight compared with common β-casein demonstrated the occurrence of genetic polymorphism at this casein locus (Chianese *et al*., 2010). Most likely due to differences in glycosylation, κ-casein is reported to be the most heterogeneous individual casein in donkey milk, including 11 components specifically immunostained using two-dimensional electrophoresis (Chianese *et al*., 2010).

Caseins exist in horse milk as large colloidal aggregates called micelles. In cow milk the stability of casein micelles is due to κ-casein, which is mainly located on their surface (Fox & Brodkorb, 2008). In horse milk, non-phosphorylated β-casein on the surface of the micelles may compensate for the lower amount of κ-casein in steric stabilisation of casein micelles (Doreau & Martin-Rosset, 2011). Although very fine yoghurt is popular, cheese cannot be obtained from horse or donkey milk. Horse milk does not form a gel during renneting but it forms fine clots, and donkey milk, although coagulable by chymosin, forms a very weak gel (Uniacke-Lowe & Fox, 2011). Differences in structure, amount and proportion of individual caseins may be responsible for the differences observed between horse, human and cow milk in micellar size (225, 64 and 182 nm, respectively) and in enzymatic, acid-induced and heat-induced coagulation (Uniacke-Lowe *et al*., 2010). The casein micellar size in donkey milk ranges from 100 to 1000 nm (Salimei, 2011).

27.4.2 Whey proteins

The main whey proteins occur in equid milk at a concentration similar to that in cow milk but with different relative percentages (Fig. 27.3). BLG, which is absent in human milk, is the most represented whey protein in both equid and cow milk. However, BLG accounts for approximately 30% of total whey proteins in horse and donkey milk, whereas in cow milk it accounts for about 50% of total whey proteins. In contrast, ALA is the predominant whey protein in human milk (Fig. 27.3).

As reviewed by Salimei and Fantuz (2012), in equid milk BLG occurs in two molecular forms (I and II); in donkey milk two variants (A and B) have been identified for

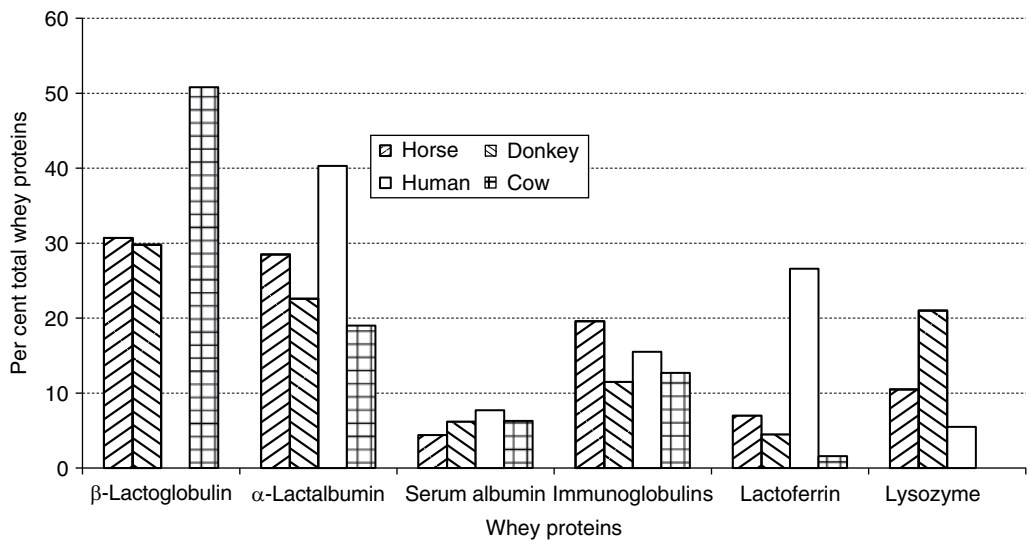


Figure 27.3. Distribution of individual whey proteins in horse, donkey, human and cow milk. Based on data from Uniacke-Lowe *et al*. (2010) and Salimei & Fantuz (2012).

BLG I, and four variants (A, B, C and D) for BLG II. In a study of 51 Ragusana donkeys, 23% of them produced milk lacking BLG II (Criscione *et al*., 2009). BLG occurs as monomers in horse milk at normal pH but in cow milk in monomeric–dimeric form (Martuzzi & Doreau, 2006).

ALA exists in horse milk in three genetic variants (A, B and C) differing by three or four amino acid replacements. In donkey milk, lysozyme occurs in at least two forms (A and B) (Uniacke-Lowe *et al*., 2010; Salimei & Fantuz, 2012). Thermal stability is higher for horse BLG and ALA than their bovine counterparts and, contrary to cows, thermal stability in horse milk is higher for BLG than for ALA (Uniacke-Lowe *et al*., 2010).

27.4.3 Non-protein nitrogen

Non-protein nitrogen (NPN) in horse and donkey milk accounts for 11% and 14% of total nitrogen, respectively (Table 27.4), which is higher than in milk from dairy ruminants (Uniacke-Lowe *et al*., 2010; Salimei, 2011). Human milk is also characterised by a high NPN content (Table 27.4). Although not completely characterised in equid milk, this fraction has potential nutritional significance for infants because it is composed, as in human milk, of urea, uric acid, creatine, creatinine, free amino acids, amino sugars and alcohols, bioactive peptides, nucleic acids and nucleotides (Emmet & Rogers, 1997). The urea content of equid milk (20–35 mg/dL), which accounts for approximately 40% of total NPN (Salimei *et al*., 2002; Fantuz *et al*., 2009a), is similar to cow milk but slightly lower than in human milk (Darragh & Lonnerdal, 2011; Salimei & Fantuz, 2012). This most likely reflects metabolic differences in urea recycling among the species.

According to Uniacke-Lowe *et al*. (2010), the concentrations of free amino acids, which are rapidly available for gut absorption, are 1960, 578 and 3020 µmol/L for horse, cow and human milk, respectively. Similarly to cow (except for threonine) and human milk, glutamic acid (568 µmol/L), glutamine (485 µmol/L), serine (175 µmol/L), threonine (137 µmol/L), alanine (105 µmol/L) and glycine (100 µmol/L) are the most represented free amino acids in horse milk. The taurine content in horse milk (32 µmol/L) is higher than in cow milk (13 µmol/L) but is approximately 10 times lower than in human milk (301 µmol/L) (Uniacke-Lowe *et al*., 2010).

27.5 FAT AND LIPID FRACTIONS IN HORSE AND DONKEY MILK

The variable fat content of horse and donkey milk (7.2 ± 8.2 and 3.4 ± 2.7 g/kg, respectively) shows a unique lipid composition, with:

- lower cholesterol content (5.0–8.8 mg/dL milk) than in cow milk but with high concentration in the milk fat;
- a higher percentage of free fatty acids (10% of fat) than in human and cow milk; and
- an average lower triglyceride (TAG) content (about 80%).

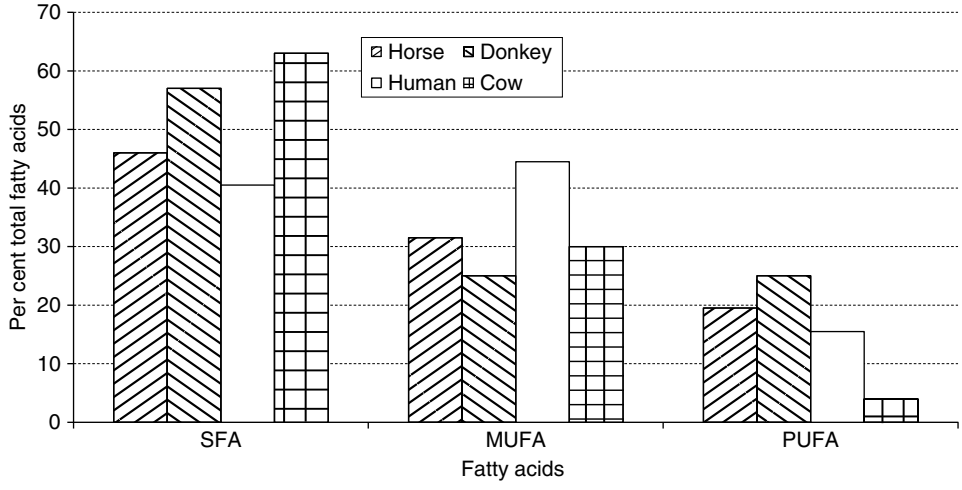


Figure 27.4. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in horse, donkey, human and cow milk. Based on data averaged from Marconi & Panfili (1998) and Salimei & Fantuz (2012).

In Fig. 27.4, the free fatty acid composition of horse and donkey milk is compared with human and cow milk. It is interesting to note that the total unsaturated fatty acid content in equid milk is closer to that in human milk, and it has a higher percentage of polyunsaturated fatty acids (PUFAs, C18 and higher). The *n*-3 PUFA content averages 8.66–11.97% of total fatty acids and 9.45–9.64% of total fatty acids for horse and donkey milk, respectively. The ratio of *n*-6 to *n*-3 PUFAs ranges from 0.57 to 1.40. Furthermore, eicosapentaenoic acid (C20:5 *n*-3), docosahexaenoic acid (C22:6 *n*-3) and arachidonic acid (C20:4 *n*-6) are reported in horse and donkey milk at levels below 0.5 g/100 g fatty acids (Salimei & Chiofalo, 2006; Salimei & Fantuz, 2012). Because of the role of these immunonutrients in both development and severity of inflammation and immune functions, horse and donkey milk could also be exploited in elderly consumers' diets. Null or small amounts of conjugated isomers of linoleic acid (CLA) have been reported in donkey and horse milk (Salimei & Fantuz, 2012), consistent with the negative relationships between CLA and polyunsaturated or monounsaturated fatty acids reported in the literature for milk from different species (Jahreis *et al*., 1999).

Dietary and nutritional factors have great influence on the variability of the milk free fatty acid profile so that feeds and feeding could be managed for dairy equids in such a way as to differentiate the nutritional and functional values of the milk products for different categories of consumers. However, the effect of dietary factors on the palatability of milk from monogastric species must also be taken into consideration, as fresh forage fed to dairy donkeys results in the green grass off-flavour (Salimei & Fantuz, 2012). Moreover, increase in soluble dietary fibre increases the percentage of saturated fatty acids and reduces *n*-6 PUFAs (Chiofalo *et al*., 2006).

When compared with milk from other dairy species, the triacylglycerol composition of horse and donkey milk shows a unique TAG profile, with high levels of C44–C52 and C44–C54, respectively (Smiddy *et al*., 2012). Human and donkey milk has a certain degree of similarity in triacylglycerol composition (Chiofalo *et al*., 2006).

In horse milk, the content of phospholipids is approximately 47 mg/L, and they are mainly composed of phosphatidylcholine (46.9%), sphingomyelin (37%), phosphatidylethanolamine (7.8%) and phosphatidylserine (5.5%) (Barello *et al*., 2008). Preliminary data on donkey milk phospholipids show a lower total content (2.94 mg/L) mainly composed of phosphatidylethanolamine (60%) and phosphatidylcholine (17.3%) (Donato *et al*., 2011). The total glycolipids in horse milk (9.5 mg/L) are characterised by glucocerebroside (55%), lactocerebroside (26%) and sulphatide (18.9%) (Barello *et al*., 2008). The content of gangliosides in horse milk is on average 1.65 mg/L (Barello *et al*., 2008).

27.6 LACTOSE AND OTHER CARBOHYDRATES IN HORSE AND DONKEY MILK

Lactose, whose principal role in milk is to provide energy to the newborn, is the major carbohydrate in equid milk and its concentration is higher than that in ruminant milk but similar to that in human milk (Fig. 27.2). Lactose synthesis in the Golgi apparatus of the mammary gland is controlled by the action of the lactose synthase complex, which contains ALA and the membrane-bound enzyme β1,4-galactosyltransferase. The concentration of lactose in milk is positively correlated to milk ALA concentration (Fox, 2011). The higher level of ALA compared with cow milk (Vincenzetti *et al*., 2008; Uniacke-Lowe *et al*., 2010) is also related to the higher lactose content of equid milk. As in milk of other species, donkey milk lactose content is inversely correlated to fat ($r = -0.61$) and protein ($r = -0.65$) content (Salimei *et al*., 2009).

In addition to lactose, mammary secretion (milk or colostrum) of most mammals contains a variety of oligosaccharides (3 to 10 monosaccharides), with lactose always located at the reducing end. More than 95% of human milk oligosaccharides are resistant to digestion and have their effect in the colon by stimulating the growth of bifidobacteria and by acting as possible anti-adhesion factors against pathogens (Urashima *et al*., 2011). The content of oligosaccharides in human milk is 12–13 g/L (22–24 g/L in colostrum) whereas goat milk contains 0.25–0.30 g/L and only trace amounts are reported for cow milk (Silanikove *et al*., 2010; Urashima *et al*., 2011). Only horse colostrum has been studied at present and a number of oligosaccharides have been identified: acidic oligosaccharides such as *N*-acetyllactosamine-α1-phosphate and 3′-*N*-acetylneuraminyllactose; and neutral oligosaccharides such as β3′-galactosyllactose (7.8 mg/L), β6′-galactosyllactose (4.8 mg/L), lacto-*N*-neotetraose, *iso*-lacto-*N*-neotetraose (0.5 mg/L), lacto-*N*-novopentaose I (1.1 mg/L), lacto-*N*-neohexaose (1.1 mg/L) and *N*-acetyllactosamine-1-O-phosphate (Uniacke-Lowe & Fox, 2011; Urashima *et al*., 2011).

In infant nutrition, sialic acid is an essential nutrient for brain development and cognition. Human milk contains a high level of sialic acid (1558, 612 and 322 mg/L, respectively, in colostrum, 1-month and 3-month milk), mostly bound to free oligosaccharides (Wang *et al*., 2001). The sialic acid content is reported to be 1333 mg/L in horse colostrum and 431 mg/L in mature horse milk (Barello *et al*., 2008), whereas lower concentrations are reported for goat milk (238 mg/L) and cow milk (62 mg/L) (Amigo & Fontecha, 2011).

27.7 MINERALS AND VITAMINS IN HORSE AND DONKEY MILK

Essential minerals (macrominerals and trace elements) in milk play a fundamental role in nutrition and health in childhood and ageing. Moreover, some macrominerals, such as calcium (Ca) and phosphorus (P), affect the stability of casein micelles (Gaucheron, 2005). Comparable concentrations of Ca, P, potassium (K), sodium (Na) and magnesium (Mg) are present in horse and donkey milk (Table 27.6), whereas higher concentrations have been reported for ruminant milk (Gaucheron, 2005; Park *et al*., 2007), concurrent with the higher ash content. However, human milk contains lower concentrations of Ca, P and Mg. Donkey milk has a slightly lower Ca/P ratio than horse milk but both are intermediate between ruminant and human milk. The relatively low content of macrominerals in equid milk can be relevant to renal functioning. Donkey milk consumption does not produce the high renal load of solutes associated with cow milk, mainly determined by the higher content of protein and inorganic substances (Iacono *et al*., 1992). The low Na content of donkey milk could represent an interesting nutritional characteristic for patients with cardiovascular concerns and hypertension. The concentration of these minerals is affected by the stage of lactation, showing a decreasing trend with advancing lactation in horse milk (Grace *et al*., 1999; Martuzzi *et al*., 2004; Summer *et al*., 2004). In donkey milk the concentrations of Ca, P and Mg, as well as the Ca/P ratio, decrease during lactation but those of K and Na show inconsistent trends (Fantuz *et al*., 2012).

Only limited and variable data are available for the trace element content of equid milk. Milk zinc (Zn) and iron (Fe) show similarities between horse and donkey species but copper (Cu) is higher in horse milk (Table 27.6). Compared with cow milk, equid milk contains higher concentrations of Fe but lower concentrations of Zn and manganese (Mn). Fe and Zn concentrations in equid milk (and Cu in horse milk) are similar to or higher than in human milk but that of Cu is lower in donkey milk (Table 27.6). Concentrations of Cu, Zn and Fe in horse milk are not affected by dietary trace element supplementation when requirements for these nutrients are already fulfilled (Grace *et al*., 1999; Kavazis *et al*., 2002).

Data on vitamin concentrations in horse milk are quite variable and only few are available for donkey milk (Table 27.7). Horse milk contains a high concentration of ascorbic acid but vitamins A and E are lower than in human milk, most likely in relation to the lower fat content of horse milk. This is particularly clear for donkey milk, which contains less fat than horse milk, and whose concentrations of vitamins A and E are also much lower than in horse milk. According to Uniacke-Lowe and Fox (2011), donkey milk contains a high vitamin B_{12} concentration but the vitamin B_3 content is approximately half that found in horse and human milk.

27.8 BIOACTIVE COMPOUNDS

Besides the nutritive role, mammary secretions contain a number of compounds that can exert biological activities. In addition to being a source of amino acids when digested, intact or partially digested (as peptide form) human milk protein and non-protein nitrogen components may play different biological roles, such as promoting the digestion and utilisation of micronutrients and macronutrients, defence

Table 27.6. Macromineral and trace element concentrations (mg/L) in horse, donkey, human and cow milk.

	Horse	Donkey	Human	Cow
Ca	500–1300	330–1140	278	970–1650
P	200–1200	320–650	140	785–1140
K	300–800	240–747	530	1340–1420
Na	167–200	100–268	180	372–534
Mg	40–110	40–83	35	92–114
Ca/P	1.72	0.93–2.37	1.7	1.23
Fe	0.22–1.46	0.43–2.64	0.72	0.3
Zn	0.9–6.4	1.23–3.19	1–3	4.0
Cu	0.2–1.0	0.08–0.30	0.2–0.4	0.09
Mn	0.01–0.05	Trace	0.003–0.006	0.03

Sources: based on data from Salimei *et al*. (2004), Gaucheron (2005), Fantuz *et al*. (2009b), Cashman (2011), Darragh & Lonnerdal (2011), Doreau & Martin-Rosset (2011) and Fantuz *et al*. (2012).

against pathogenic bacteria and viruses, immunomodulation, metabolism and growth regulation (Lönnerdal, 2003). These bioactive compounds are active at the mammary gland level but also at intestinal and systemic levels in breast-fed infants (Lönnerdal, 2010).

Among proteins with antimicrobial activity, horse and donkey milk contains high levels of lysozyme (about 1 or 4 g/L in chemical or microbiological assays, respectively) (Coppola *et al*., 2002; Vincenzetti *et al*., 2008; Doreau & Martin-Rosset, 2011) and lactoferrin compared with cow milk. Lysozyme in equid milk is resistant to thermal treatments (Coppola *et al*., 2002; Di Cagno *et al*., 2004) and to digestion by acids, proteases (Uniacke-Lowe *et al*., 2010) or human gastrointestinal enzymes (Table 27.8). The antimicrobial activity of donkey milk is also a result of peptides being released during the gastrointestinal digestion of milk protein (Nazzaro *et al*., 2010; Tidona *et al*., 2011). One peptide (derived from β-casein) with angiotensin-converting enzyme (ACE)-inhibitory activity has been identified by simulating gastrointestinal digestion of donkey milk (Bidasolo *et al*., 2012). Moreover, compounds which exert an antiproliferative and antitumor effect *in vitro* have been recently observed in donkey milk (Mao *et al*., 2009).

Table 27.7. Vitamin concentrations (mg/L) in horse, donkey, human and cow milk.

	Horse	Donkey	Human	Cow
Vitamin A	0.093–0.34	0.017	0.3–0.7	0.3–0.5
Vitamin D	0.003		0.0004	0.003
Vitamin E	0.26–1.13	0.051	3–8	0.98–1.28
Vitamin K	0.029		0.003–0.015	0.011
Vitamin C	17.2–147		50–100	21
Thiamin, B_1	0.30	0.41	0.15	0.37
Riboflavin, B_2	0.37	0.64	0.4–0.6	1.8
Niacin, B_3	1.4	0.74	1.7	0.9
Pantothenic acid, B_5	3.0		2.7	3.5
Piridoxine, B_6	0.30		0.14	0.64
Cobalamin, B_{12}	0.003	1.1	0.50	0.004

Sources: based on data from Csapò *et al*. (1995), Marconi & Panfili (1998), Sorrentino *et al*. (2005), Darragh & Lonnerdal (2011) and Uniacke-Lowe & Fox (2011).

Horse and donkey milk contains bioactive compounds whose determination, using human peptides as standard and specific antibodies (radioimmunoassay and ELISA), has been validated for the equid species. Among the human-like growth factors and hormones, leptin (3.35–5.32 ng/mL) has been identified in both horse and donkey milk (Salimei *et al*., 2002, 2005b, 2007), while insulin-like growth factor 1 (9.81–13.50 ng/mL) and triiodothyronine (4.0 ng/mL) have been measured only in donkey milk (Magistrelli *et al*., 2008; Todini *et al*., 2010). Such molecules play a role in regulating the infant's food intake, metabolism and body condition (Lönnerdal, 2010), but the activities *in vivo* of such milk molecules remain to be verified. Dietary supplementation with trace elements (including iodine) does

Table 27.8. *In vitro* digestion of horse, donkey, human and cow milk proteins. Data are expressed as percentage of undigested protein (100 at the beginning of the process) after incubation with human gastric juice (step 1; pH 2) and intestinal juice (step 2).

		Horse	Donkey	Human	Cow
Caseins	Step 1	30	60	39	69
	Step 2	4	6	5	4
β-Lactoglobulin	Step 1	104	89	*	102
	Step 2	25	30		64
α-Lactalbumin	Step 1	104	105	97	100
	Step 2	93	95	92	91
Serum albumin	Step 1	61	73	79	64
	Step 2	15	23	34	26
Lactoferrin	Step 1	42	38	31	43
	Step 2	8	6	0	6
Lysozyme	Step 1	112	101		
	Step 2	64	75		

*Not present.

Sources: based on data from Inglingstad *et al*. (2010) and Tidona *et al*. (2011).

increase triiodothyronine concentration in donkey milk (Todini *et al*., 2012).

27.9 HORSE AND DONKEY MILK IN THE HUMAN DIET AND WELL-BEING

Because of their compositional peculiarities, horse and donkey milk has been used in human nutrition since ancient times, in particular as a cow milk substitute and as food with health-promoting properties. Advances in knowledge of horse and donkey milk have renewed the interest in this unconventional source of food for sensitive consumers, such as people suffering from allergies and elderly consumers (Salimei & Fantuz, 2012).

27.9.1 Equid milk sanitation and quality standards and controls

Besides specific knowledge of its nutritional and allergenic profile, raw equid milk requires an appropriate evaluation in terms of hygiene and health. The total bacterial count of equid milk ranges from 250 to 741 000 cfu/mL, emphasising the importance of hygiene in milking and handling raw equid milk. Notwithstanding the natural antimicrobial activity, due to the presence of functional and bioactive compounds, lactic acid bacteria as well as Enterobacteriaceae, coliforms, yeasts and moulds grow well in donkey milk (Colavita *et al*., 2011). No *Listeria monocytogenes* and *Salmonella* spp. have been found in horse or donkey milk; however, they are considered among the undesirable microbiota along with *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and the pathogens *Brucella* spp., *Rhodococcus equi*, *Streptococcus equi* subsp. *zooepidemicus*, *Streptococcus dysgalactiae* subsp. *equisimilis*, *Mycobacterium avium* complex, *Campylobacter* spp., *Yersinia enterocolitica* and *Clostridium difficile* (Colavita *et al*., 2011).

In addition to European rules on safety of raw milk (EC 852 and 853/04), state members are introducing specific requirements at regional levels for selling raw donkey milk. However, there is no homogeneity in the quality standards, with total bacterial counts ranging from 25 000 to 500 000 cfu/mL (Colavita *et al*., 2011). As for raw cow milk, other microbiological parameters and criteria need to be satisfied in raw donkey milk for human consumption (EC 1441/07); no limits are reported for somatic cell count in raw donkey milk. Plans for brucellosis control are required for authorised dairy donkey farms (Colavita *et al*., 2011). Because of the strict EC regulations and because of the small number of dairy equids, the consumption of raw horse and donkey milk is associated with a lower microbial risk than for bovine milk, when stored at 4°C for a maximum of 3 days and consumed immediately after heating (Colavita *et al*., 2011).

It is important to consider that some functional compounds naturally present in milk are irreversibly damaged by the heat treatments necessary for milk sanitation and/or prolonged shelf-life. Therefore it must be remembered that there is significant modification of the nitrogenous profile of donkey milk after heating at 90°C for 1 min (Fig. 27.5); in addition, among the fat-soluble nutrients, the α-tocopherol content decreases with increasing temperature and duration of heat processing (Sorrentino *et al*., 2005).

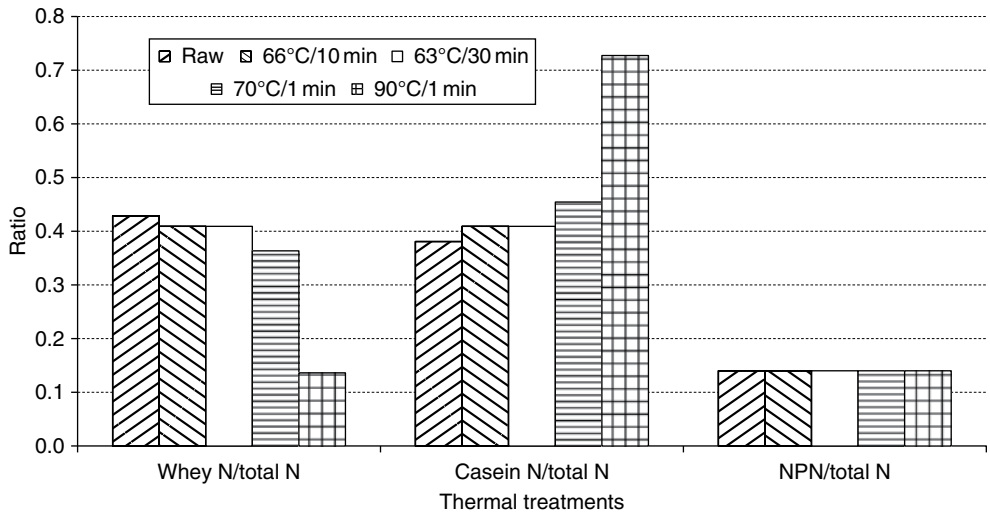


Figure 27.5. Distribution of nitrogen (N) fractions in donkey milk, raw and after thermal treatments. Based on data from Sorrentino *et al.* (2005).

An elevated lipase activity and the possible lipid peroxidation of unsaturated fatty acids (Doreau & Martin-Rosset, 2011) should also be considered in equid milk processing and packaging.

A molecular marker of thermal and storage damage in milk, furosine (ε-*N*-2-furoylmethyl-L-lysine), has been found in heat-treated donkey milk (90°C, 1 min) at a concentration of 112.9 mg/100 g protein (Sorrentino *et al*., 2005). Much higher values have been observed in commercial powdered horse milk, as well as for the ratio of *cis*-retinol to *trans*-retinol, while nutrients such as tocopherols and vitamin C have been found partially destroyed (Marconi & Panfili, 1998). Moreover, the lysine content of powdered horse milk was found to be lower than in raw or freeze-dried milk (Marconi & Panfili, 1998). With the increasing concern about the persistence of allergenicity in pasteurised cow milk and the effect of heat processing on milk protein sensitisation of consumers (Restani *et al*., 2009), optimal technological processes and storage conditions need to be defined in order to preserve the unique functional components of equid milk.

27.9.2 Horse and donkey milk as hypoallergenic and functional food

Cow milk protein allergy is a food allergy that affects 3% of children in the first 3 years of life, but can also be retained for life (El-Agamy, 2007; Restani *et al*., 2009). There is growing interest in alternative food sources for children suffering from cow milk allergy. Commonly available hypoallergenic milk formulae are made with soy or cow milk proteins hydrolysed to different extents (Restani *et al*., 2009), but such formulae are in some cases rejected due to low palatability, cross-reactions, etc. (El-Agamy, 2007). Clinical studies indicate that, when adequately supplemented in fat and energy, horse and donkey milk can be successfully used for children suffering IgE- and non-IgE-mediated cow milk allergy (Fig. 27.6). A proper nutritional balance must be carefully considered (Tesse *et al*., 2009; D'Auria *et al*., 2011); however, Iacono *et al*. (1992) fed allergic children 210–250 mL fat-supplemented (4 mL vegetable oil per dL) donkey milk per kilogram body weight per day, reporting satisfactory clinical conditions and growth rate that continued in the follow-up. This represents the first scientific evidence on the use of donkey milk in childhood nutrition after D'Arval's initial observation (D'Arval, 1912). Equid milk has high palatability, and the taste of horse and donkey milk can be appealing to children due to the high lactose content (Salimei, 2011).

Different factors may be responsible or concurrent for the hypoallergenic properties of donkey and horse milk, one of them being the ratio of casein to whey protein, which is more similar to human milk (Table 27.4). The casein/whey protein ratio is reported to affect the allergenicity of cow milk (Lara-Villoslada *et al*., 2005). In this

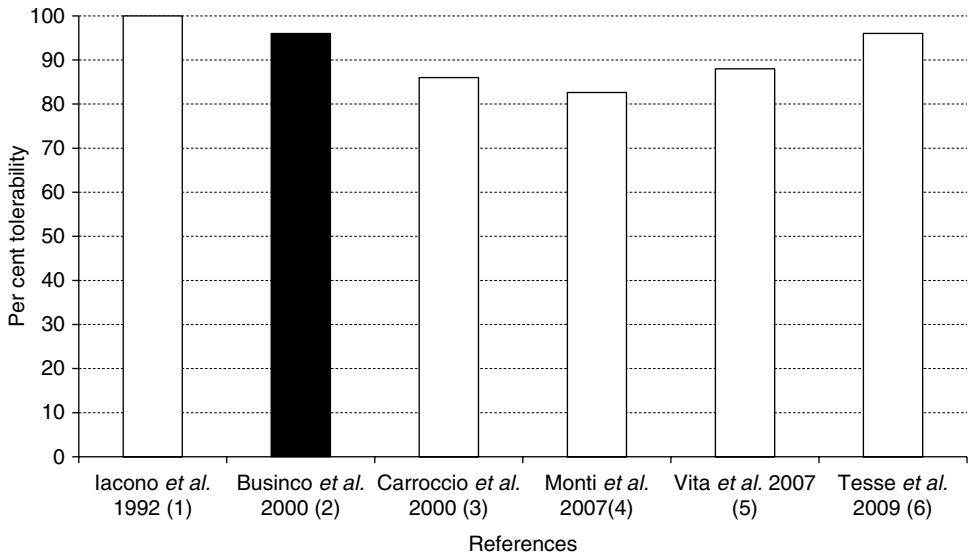


Figure 27.6. Clinical studies on tolerability to horse (■) and donkey (□) milk of children with food allergy. (1) nine unweaned children (26–79 days); (2) 25 children (19–72 months); (3) 21 children (10 days to 9 months); (4) 46 children (12–149 months); (5) 26 children (6 months to 3 years); (6) 30 children (6 months to 11 years). Based on data from the reference sources shown in the diagram.

regard, patients who tolerated equine milk experienced intolerance to goat or sheep milk, characterised on average by a casein to whey protein ratio of 3–4 : 1 (Uniacke-Lowe *et al*., 2010). Although different milk proteins may be responsible for allergic reactions, BLG and casein are the main allergens in cow milk (El-Agamy, 2007). The hypoallergenic properties of equid milk may be related to the differing amounts and proportions of individual caseins and whey proteins among species (Uniacke-Lowe *et al*., 2010).

The fact that gastrointestinal digestion may affect the allergenic properties of milk cannot be excluded. Table 27.8 shows that after 30-min incubation with human gastric juice, followed by 30-min incubation with human duodenal juice, caseins were the most digested fraction, followed by lactoferrin and serum albumin in horse, donkey, human and cow milk. However, the percentage of undigested horse and donkey BLG was approximately half its bovine counterpart, so that a lower amount of potential allergen would be available for absorption by the intestinal mucosa.

Differences in protein primary structures due to phylogenetic origin (Table 27.9) may also affect milk protein allergenicity. According to Cunsolo *et al*. (2011), the remarkable differences in the amino acid sequence of the IgE-binding linear epitopes between cow and donkey α_{s1}-casein may be responsible for the hypoallergenic properties of donkey milk.

As reviewed by Doreau and Martin-Rosset (2011), the scientific data on the health properties of horse milk are not always reliable; however, it has been used for the treatment of humans suffering from diseases such as chronic hepatitis and peptic ulcers as well as anaemia and nephritis. In the treatment of tuberculosis, horse milk consumption increases the number of red blood cells and lymphocytes (Doreau & Martin-Rosset, 2011). Horse milk is increasingly used as an alternative medicine for the treatment of psoriasis and atopic eczema (Gall *et al*., 1996). Horse milk has also been used, raw or fermented, in the treatment of digestive diseases, in which easier digestion in the stomach may play a role. Horse caseins form a soft coagulum that leaves the stomach within about 2 hours, whereas cow caseins form firm clots that leave the stomach within about 3–5 hours (Uniacke-Lowe & Fox, 2011). Moreover, horse caseins are more susceptible to *in vitro* digestion by human gastric juice than cow and donkey caseins (Table 27.8).

The use of donkey milk is also reported to be useful in the prevention of atherosclerosis and to exert beneficial effects on immune response in healthy elderly people (Amati *et al*., 2010). In this regard, the low milk fat content and the nutritionally relevant free fatty acid components, with low atherogenic (~0.80) and thrombogenic (~0.32) indices, support the consumption of donkey milk by elderly consumers (Salimei, 2011).

With regard to adult consumption of equid milk, one case of IgE-mediated horse milk allergy has been reported in the literature, most likely caused by heat-labile ALA and BLG, which do not cross-react with corresponding whey proteins in cow milk (Gall *et al*., 1996). Furthermore, relationships between inhalant sensitisation to dander allergens and milk protein allergens have been reported for both horse and donkey (Salimei & Fantuz, 2012).

27.9.3 Equid milk dairy products

Because of its nutritional and microbiological characteristics, horse and donkey milk and their derivatives can be considered as foods with interesting health-promoting properties. Equid milk is not suitable for cheese production but traditional fermented horse milk beverages (*airag* and *koumiss*) are very popular in Eurasian steppe areas, and more recently in some European countries (Uniacke-Lowe, 2011). *Koumiss*, the name probably derived from the Kumanese society, has been known since 2000 BC in Central Asia and spread through nomadic Scythians and other ancient populations, and is still produced by Kazakhs, Kirghizes, Bashkirs, Yakuts, Kalmuks, Bouriates and Mongols (Langlois, 2010). In particular, *airag* is the national drink in Mongolia (per-capita consumption, 50 L/year) and

Table 27.9. Percentage homology of some horse, donkey and human milk proteins with their bovine counterparts (100) and, in parentheses, percentage homology between horse and human individual caseins.

	Horse	Donkey	Human
α_{s1}-Casein	43.3 (54)	60.0	31.9
β-Casein	60.5 (59)	Not available	56.5
κ-Casein (%)	57.4 (65)	Not available	53.2
β-Lactoglobulin	59.4	56.9–51.6	Not present
α-Lactalbumin	72.4 A; 69.1 B/C	71.5	73.9
Serum albumin	74.5	74.1	76.6

Sources: based on data from Martuzzi & Doreau (2006) and Restani *et al*. (2009).

has high social importance (Langlois, 2010; Uniacke-Lowe, 2011). The traditional *koumiss* is a sour-tasting, alcoholic, fermented drink whose therapeutic roles in the treatment of digestive, urogenital and cardiovascular diseases and tuberculosis, and during oncological treatments have been reviewed by Uniacke-Lowe (2011) to be more effective than raw horse milk. On an annual basis, sanatoriums in Kazakhstan, Kyrgyzstan and Crimea use about 2 million, 100 000 and 140 000 L of *koumiss*, respectively. Mongolia produces about 13.5 million litres of *airag*. The *koumiss* microbiota was identified approximately 50 years ago and comprises *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus casei*, *Lactococcus lactis* subsp. *lactis*, *Kluyveromyces fragilis* and *Saccharomyces unisporus* (Uniacke-Lowe, 2011). However, molecular biology-based studies on the diversity of lactic acid bacteria (LAB) and yeasts in *airag* show a unique microbial composition, including *Lactobacillus helveticus*, *Lactobacillus kefiranofaciens* and *Kluyveromyces marxianus* among predominant LAB and yeast species (Watanabe *et al*., 2008), indicating microbial differences among traditional fermented products from horse milk. Furthermore, morphological, biochemical and molecular characterisations have been carried out on *airag*/*koumiss* microbiota, leading to the discovery of the new species *Bifidobacterium mongoliense* (Watanabe *et al*., 2009) and *Lactobacillus casei* Zhang (Guo *et al*., 2009) with potential probiotic and health-promoting properties. Strains of LAB isolated from *koumiss* have a cholesterol-reducing effect (Pan *et al*., 2011) and antifungal properties (Wang *et al*., 2011). In *koumiss*, bacteriocins from LAB have been isolated and novel ACE-inhibitory peptides have been identified but their activities *in vivo* remain to be verified (Salimei & Fantuz, 2012).

Non-traditional *koumiss* is also produced according to standardised manufacturing protocols, and these have been recently revised in order to enhance the rheological and sensorial characteristics of fermented milk, as described by Uniacke-Lowe (2011). Based on lactic acid and ethanol content, mild (0.6–0.8% acidity, 0.7–1.0% ethanol), medium (0.8–1.0% acidity, 1.1–1.8% ethanol) and strong (1.0–1.2% acidity, 1.8–2.5% ethanol) types of *koumiss* are produced (Uniacke-Lowe, 2011). The lowered lactose content (4.5–5.5%) of *koumiss* compared with raw horse milk should be considered by lactose-intolerant consumers (Uniacke-Lowe, 2011) but sensorial characteristics could be a limit to the diffusion of fermented horse milk. Moreover, cultural constraints related to refusal of unconventional milk, with neglect of the nutritional value, could interfere with consumer acceptability.

Thanks to the low microbial population and the high lysozyme content, raw donkey milk has also been successfully used as a base ingredient in probiotic beverage production (Coppola *et al*., 2002; Chiavari *et al*., 2005). In addition, ice cream, biscuits, cakes and pudding are produced in Italy on an artisanal scale from pasteurised donkey milk (Salimei, 2011) and may represent an innovation in the dairy food scenario.

27.10 CONCLUSIONS

Horses and donkeys have been known as dairy species since ancient times, even though the domestication of these equid species as a source of animal power is mainly related to the development of rural societies in the far past. Fermented horse milk is traditionally produced in Eurasian steppe areas where *koumiss* or *airag* is a dietary ingredient of specifically traditional eating habits.

Thanks to the lactose content and the unique nitrogenous and lipid composition, horse and donkey milk and derivatives, fermented or not, can be considered interesting foods for allergic infants and the elderly. However, equid milk consumption by sensitive consumers should be clinically tested and the diet should be supervised by a nutritionist.

As supported by the relevant scientific literature on raw and fermented equid milk, the claimed health-promoting properties are related to the presence of functional and bioactive compounds derived from animal metabolism or released during the digestive process or fermentation by microorganisms. In the dairy food chain for sensitive consumers, the management of horse and donkey nutrition and their milk must be carefully regulated and evaluated with specific traceability protocols concerning food safety.

Apart from the traditional production of horse milk in Eurasian regions, this innovative food chain is rapidly carving a niche in Mediterranean countries, where animal power has been replaced by mechanisation and most equid breeds are endangered. The dairy horse and donkey enterprise is mainly an activity of multifunctional farming systems in marginal or low-productivity areas around the world, which would otherwise be destined for depopulation with serious consequences for the environment.

As a final remark, donkeys are mostly present in regions of Africa, Asia and Latin America and donkey milk production may help increase the availability of dietary animal proteins, especially for infants where breastfeeding is not possible or advisable.

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28
Sow Milk

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28.1 INTRODUCTION

A domestic pig is highly prolific, typically giving birth to 10–16 piglets. A sow therefore produces an ample amount of milk to support the growth of her litter. A sow produces as much milk per kilogram body weight as a dairy cow. Milk is the sole source of nutrients to nursing piglets. Growth and health of nursing piglets therefore depend entirely on the quantity and quality of milk from a sow (Kim *et al*., 2000, 2009). Improving milk production and quality is an important issue in swine production as it is closely related to weight gain of piglets. High mortality of nursing piglets is associated with low milk production during the first few days after birth (de Passille & Rushen, 1989). Hypoglycemia and death due to low milk production is not uncommon in neonatal pigs (de Passille & Hartsock, 1979).

Milk synthesis occurs in a mammary epithelial cell and thus the number of mammary cells is closely related to the milk production potential of a sow (Tucker, 1987; Kim *et al*., 2000). Mammary gland growth is closely affected by maternal nutrition (Kim *et al*., 1999a). Therefore, achieving maximal quantity and quality of sow milk through adequate sow nutrition during gestation and lactation improves survival and growth of the offspring (Miller *et al*., 1994). Enhancing milk composition and production to optimize growth and health of piglets is of great importance to pig producers. Milk is a pivotal factor in piglet survival, growth, health, and body composition prior to weaning, as would also be applicable to the situation in human infants. From a practical point of view, therefore, the same concept of maternal nutrition and milk quality related to growth and health of piglets can also hold great value for scientific research into human nutrition, medicine, and food science (Park, 2006). In this chapter, the structure and growth of porcine mammary glands, the characteristics of porcine milk and colostrum, and their relation to the growth and health of nursing piglets are discussed.

28.2 PORCINE MAMMARY GLAND

28.2.1 Structure and anatomy

Sows usually have 12–18 mammary glands, with the number of teats ranging from 6 to 32 (Turner, 1952; English *et al*., 1977; Kim *et al*., 2000). Additional teats are often classified as supernumerary and rectal (Pond & Houpt, 1978). Mammary glands are arranged in two parallel rows, one each side of the ventral midline. Each mammary gland usually has two separate glandular systems, each with a lactiferous duct which leads to a single opening (Cooper, 1840; Turner, 1952). Cisternae of the fully developed mammary gland are relatively small compared with ruminants (Turner, 1952). The porcine mammary gland contains a large number of "sack-like" globular alveoli that secrete milk (Fig. 28.1). The duct system drains the lumen of the alveolus. Connective tissue integrates each alveolus. A cluster of alveoli is organized to form a lobule (Turner, 1952). The alveoli contain a single layer of epithelial cells that synthesize the major components of milk and secrete their contents into the lumen of the alveolus during lactation (Larson, 1985). Epithelial cells are surrounded by myoepithelial cells longitudinally above the basal surface of the epithelial cells (Anderson, 1985).

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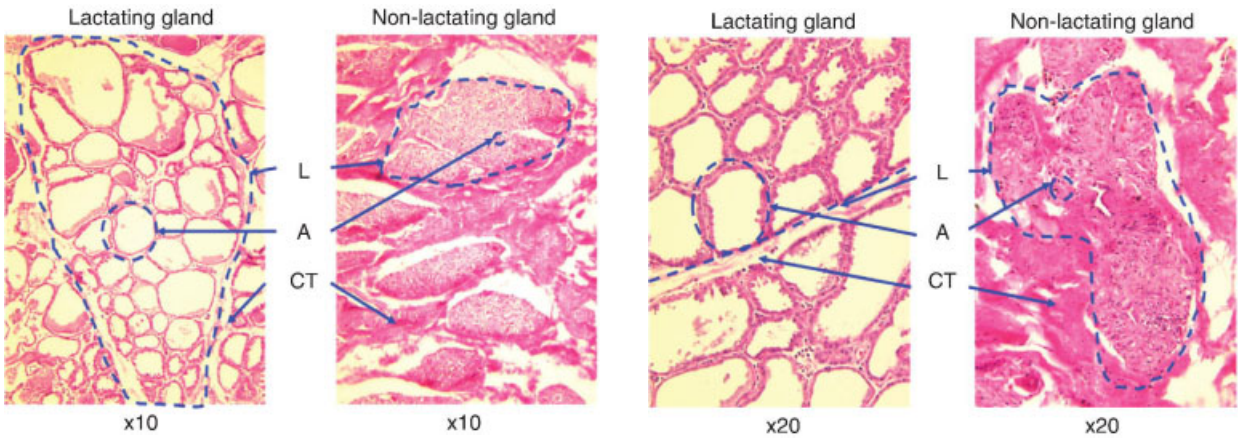


Figure 28.1. Structure of porcine mammary gland. L, lobule; A, alveoli; CT, connective tissue. (Left) ×10. (Right) ×20. Based on Kim (1999). For a color version of this figure, see Plate 28.1.

Myoepithelial cells function as contractile units for the ejection of milk from the lumen of the alveolus into the ducts. The newly synthesized milk is retained in the lumen of alveoli and secreted to piglets after an oxytocin surge (Boyd *et al*., 1995). Thus, the number of mammary cells and the amount of nutrients available to those mammary cells are the critical determinants of milk production.

Blood is provided to the mammary glands through two major arteries. The anterior mammary glands receive blood from branches of the right and left brachial (carotid) arteries, while the posterior mammary glands receive blood from a branch of the abdominal aorta (Turner, 1939; Hartmann & Hughes, 1996). Anterior and posterior mammary veins are two separate routes for the return of blood from the mammary glands to the heart. Oxygen and nutrients are supplied to epithelial cells and myoepithelial cells through a dense capillary system.

28.2.2 Mammary gland growth

Mammary gland growth in gestating sows has been characterized in few papers. Mammary glands undergo physiological and morphological changes at the onset of pregnancy and grow substantially during gestation. Previous research has demonstrated that the size of mammary glands in a multiparous sow increases mostly during days 75–90 of gestation (Hacker & Hill, 1972; Kensinger *et al*., 1982) and increases almost fourfold during gestation as indicated by DNA content. Using a lean type sow, Ji *et al*. (2006) investigated mammary gland growth in pregnant gilts and showed that the size of mammary glands increases 24-fold during gestation as indicated by protein content in mammary parenchymal tissue (Fig. 28.2). This is a gain of about 50 g protein per gland in a pregnant gilt (Kim *et al*., 2009). During

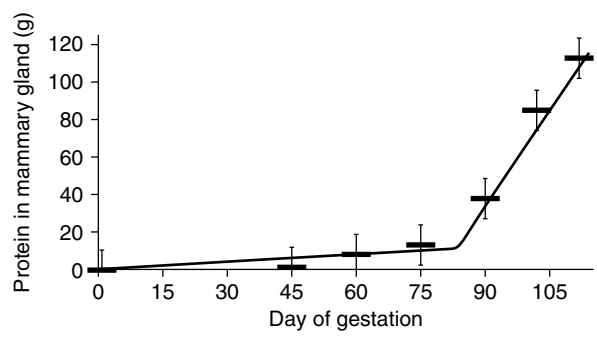


Figure 28.2. Protein content (g per gland) in mammary gland during gestation. Protein content in an individual mammary gland was increased by 0.41 g/day up to day 80 of gestation, whereas it increased to 3.41 g/day from day 80 of gestation to farrowing. Increase in protein from day 80 of gestation is greater ($P<0.05$) than increase in protein up to day 80 of gestation. Based on Ji *et al*. (2006) and Kim *et al*. (2009).

gestation, mammary glands undergo compositional changes: the percentage of fat decreases, whereas the percentage of protein and DNA increases during lactation (Fig. 28.3).

Compositional changes occur in mammary glands during gestation and lactation (Kim *et al*., 1999b; Ji *et al*., 2006). The percentage of dry matter in fresh mammary parenchymal tissue decreases from 74% at breeding to 40% prior to farrowing and further decreases from 32% on farrowing to 24% at weaning. The percentage of protein in dried mammary parenchymal tissue increases from 7% at breeding to 38% prior to farrowing and further increases

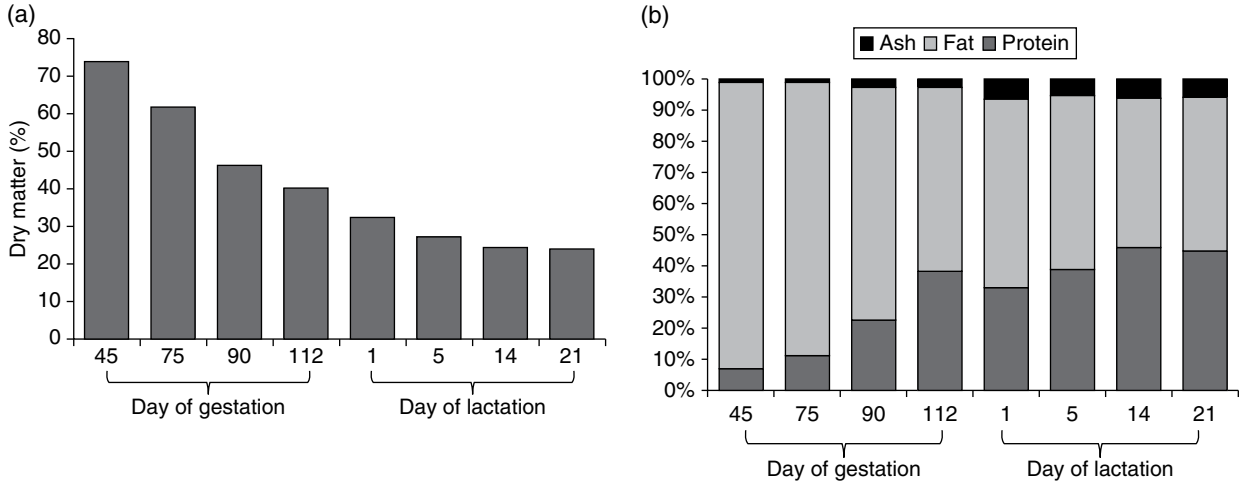


Figure 28.3. Per cent composition in mammary gland during gestation and lactation. (a) Per cent composition of dry matter. Based on Kim *et al.* (1999b). (b) Per cent composition of protein, fat, and ash in dry matter. Reproduced from Ji *et al.* (2006), with permission of the American Society of Animal Science.

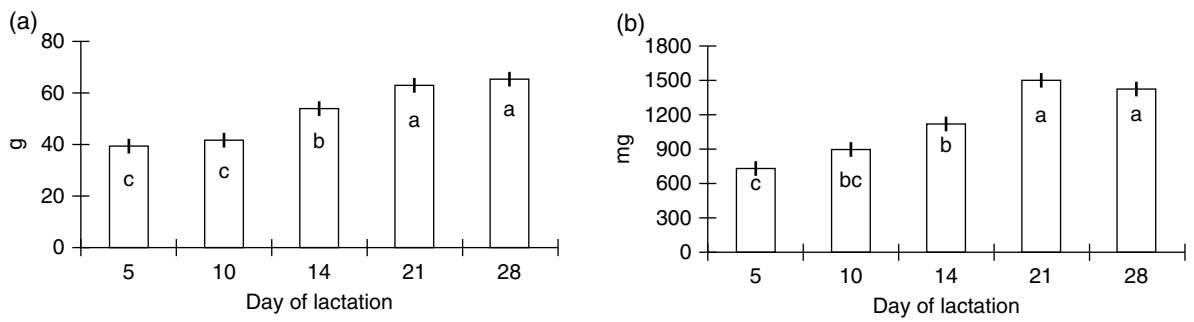


Figure 28.4. Contents of (a) protein (g per lactating gland) and (b) DNA (mg per lactating gland) in suckled mammary glands during lactation. Bars lacking a common lower case letter differ ($P<0.05$). Based on Kim *et al.* (1999b).

from 39% on farrowing to 47% at weaning. The percentage of fat in dried mammary parenchymal tissue decreases from 92% at breeding to 47% at weaning (Fig. 28.3). These compositional changes are caused by structural changes as a greater proportion of the tissue is composed of adipocytes and connective tissues at early gestation, which is replaced by alveoli as gestation progresses (Ji *et al.*, 2006).

After farrowing, newborn piglets find mammary glands using unique behavior to select a teat (McBride, 1963; Hartsock & Graves, 1976; Rohde-Parfet & Gonyou, 1990). Piglets start to form a unique teat order within a few days after birth. Therefore, litter size usually matches the number of lactating mammary glands. Suckled mammary glands continue to grow during lactation whereas unsuckled mammary glands undergo rapid involution. Kim *et al.* (1999b) used a lean-type sow and demonstrated that suckled mammary glands increase their size 1.65-fold or 1.94-fold when measured by protein or DNA content, respectively (Fig. 28.4). This indicates that continuous growth of mammary glands during lactation is important for the increase in milk yields. During lactation, suckled mammary glands undergo compositional changes. The percentage of fat decreases, whereas the percentage of protein and DNA increases during lactation.

Unsuckled mammary glands undergo rapid involution. Kim *et al.* (2001a) showed that an unsuckled mammary gland lost 25, 55, and 65% of protein in mammary parenchymal tissue by 3, 6, and 9 days after farrowing, respectively, and then remained the same until weaning (Fig. 28.5). Thus when cross-fostering piglets among litters, it is important to do it within 3–4 days after farrowing in light of the rapid involution of unsuckled mammary glands.

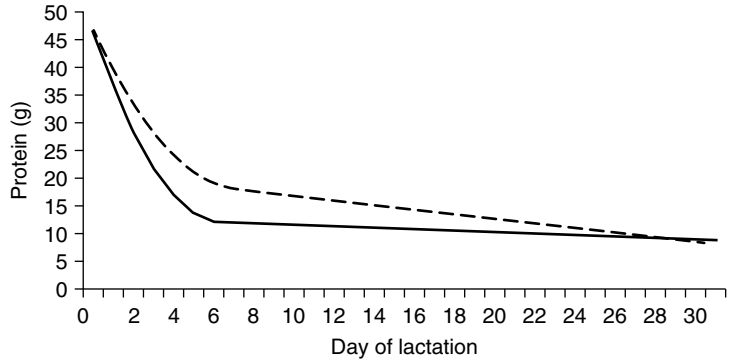


Figure 28.5. Protein content (g per gland) in unsuckled mammary glands during lactation. Solid line indicates unsuckled mammary glands from sows fed 12 Mcal metabolizable energy and 32 g lysine per day. Dashed line indicates unsuckled mammary glands from sows fed 17.5 Mcal metabolizable energy and 65 g lysine per day. Protein content in unsuckled mammary glands reduced ($P<0.05$) by day 2 of lactation and reached the minimum value by day 6 of lactation. Reproduced from Kim *et al.* (2001a), with permission of the American Society of Animal Science.

The growth of the mammary gland is affected by its anatomical location on a sow. Earlier studies indicated that lactating mammary glands in anterior location are larger than others (Donald, 1937; Gill & Thomson, 1956). However, it is interesting to note that mammary glands in the middle part of the body grow faster during gestation and bigger in size at farrowing compared with mammary glands in anterior and posterior locations on a sow (Ji *et al.*, 2006). This may be because there is more physical space for mammary glands to grow in the middle part of the body whereas anterior and posterior locations are hindered by the legs. It is also speculated that blood supply starts from the middle location, typically the third mammary glands, and then extends to the front and back of the body and thus mammary glands in the middle location have a greater chance of obtaining nutrients compared with those in other locations. During lactation, however, the growth rates of mammary glands by location change compared with growth rates during gestation (Kim *et al.*, 2000). Mammary glands in the middle location are larger than others at farrowing but anterior mammary glands grow faster than others during lactation (Fig. 28.6). This may be because anterior mammary glands are preferred by piglets during lactation (Fig. 28.7). Growth of suckling piglets was greater when they suckled the first five pairs of mammary glands compared with posterior mammary glands (Fig. 28.8). Posterior mammary glands had greater variation in their sizes and milk production whereas anterior and middle mammary glands were more uniform in size and milk production (Kim *et al.*, 2000).

During lactation, milk removal is the most important factor in maintaining lactation and mammary gland growth. Milk components accumulate in the alveolar lumen as milk is secreted from the epithelial cells. An autocrine factor, termed the feedback inhibitor of lactation (FIL; Wilde *et al.*, 1995; Knight *et al.*, 1998), also accumulates in the alveolar lumen, which inhibits further milk secretion from the cells. Suckling removes the inhibitory effect of FIL and allows continued milk secretion. If milk removal does not occur over a period of a couple of days, then mammary gland involution begins. This is accompanied by a decline in secretion of galactopoietic hormones involved in maintaining active epithelial cells. Other hormones, particularly estrogen, may affect mammary involution. Mammary involution is characterized by substantial morphological and histological changes in the tissue, changes in the composition of mammary secretions, and loss of lactating epithelial cells (Hurley, 1989). Mammary involution occurs during the initial 7 days after weaning, with irreversible significant changes occurring by day 2 after weaning (Ford *et al.*, 2003). Cross-sectional area of mammary parenchymal tissue decreases by over 55% in the first week after weaning. During the period of involution, the sow's mammary gland loses over two-thirds of its parenchymal mass and nearly two-thirds of the cells that were present on the day of weaning. Mammary glands that are not suckled during lactation do not have further significant loss of parenchymal tissue during the first 7 days after weaning.

28.2.3 Maternal nutrition and mammary gland growth

In a production setting, sows are allowed limited access to feed during gestation in order to control calorie intake and so prevent obesity at farrowing. However, restricted

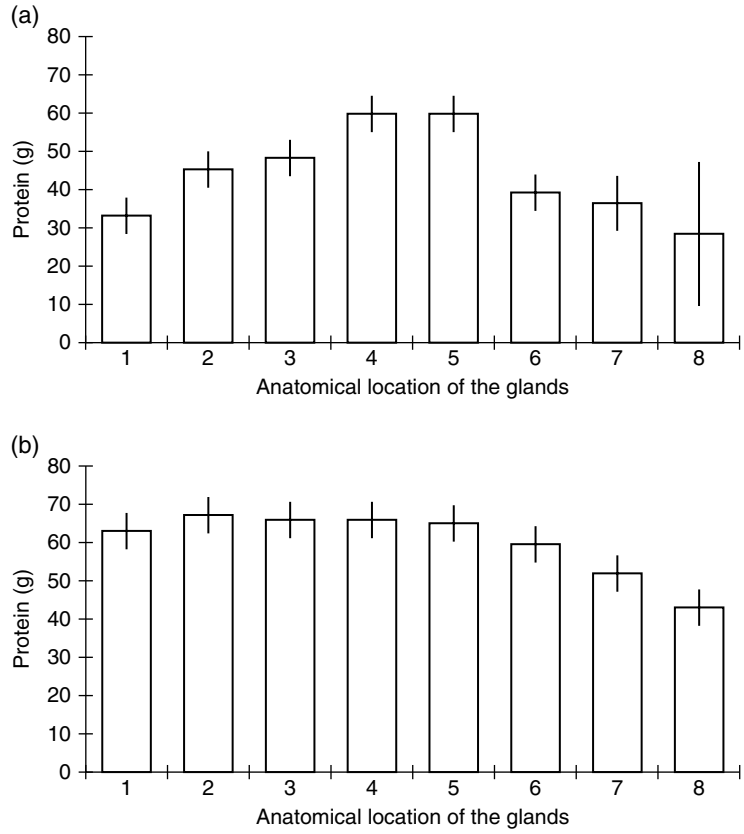


Figure 28.6. Protein content (g per gland) in suckled mammary glands by anatomical location within 12 hours post farrowing (a) and on day 21 of lactation (b). At farrowing, fourth and fifth pairs of mammary glands had more ($P<0.05$) protein than others, whereas the first five pairs of mammary glands had more ($P<0.05$) protein than others by the end of lactation at 3 weeks after farrowing. Based on Kim *et al.* (2000).

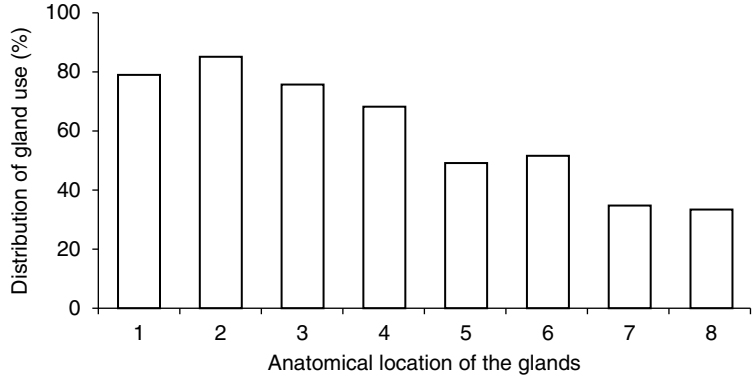


Figure 28.7. Suckling preference of mammary glands by piglets. Reproduced from Kim *et al.* (2000), with permission of the American Society of Animal Science.

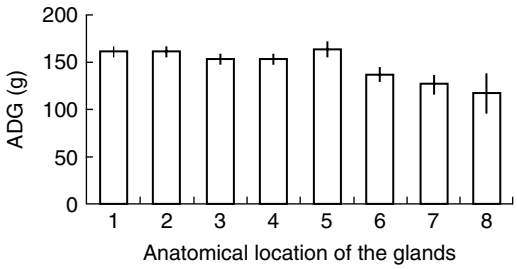


Figure 28.8. Growth of piglets suckling mammary glands by anatomical location. Average daily gain (ADG) of piglets suckling the first five pairs of mammary glands was greater ($P<0.05$) than those suckling the posterior mammary glands. Based on Kim *et al.* (2000).

feeding can cause protein deficiency especially during late gestation. Ji *et al.* (2006) have demonstrated that the protein gain in mammary parenchymal tissues increases 24-fold during late gestation (3.41 g/day from day 80 of gestation) compared with early to mid gestation (0.14 g/day up to day 80 of gestation), indicating increases in nutrient requirements for mammary gland growth during late gestation (Fig. 28.2). A large difference in the rate of protein gain in each gland by stage of gestation indicates that a sow will have increased nutrient supply to support the growth of mammary glands especially during late gestation. If a sow has 15 mammary glands, protein accretion in mammary tissue is 2 g/day and this increases to 51 g/day from day 80 of gestation. To support the additional gain of 49 g/day of protein, dietary needs for additional protein will be significantly high.

Mammary tissue growth continues in suckled glands during lactation in sows (Kim *et al.*, 1999b). For example, wet weight of individual suckled mammary glands increases by 55% and total gland DNA doubles during 4 weeks of lactation. This growth requires 6 g/day true ileal digestible lysine, as there is 1 g/day lysine increase in mammary tissue and 5 g/day lysine used for maintenance of mammary tissue (Trottier *et al.*, 1997; Kim *et al.*, 1999b). Therefore, nutrient requirements for mammary gland growth should be considered when feeding lactating sows. Insufficient nutrient intake caused by low voluntary feed intake impedes a sufficient supply of nutrients to mammary tissue for growth and development. Interestingly, the amount of amino acids taken up by the mammary gland does not change (Trottier *et al.*, 1997), although the number of its epithelial cells increases (Kim *et al.*, 1999a) with advancing stages of lactation, indicating a diminished rate of amino acid transport by these cells.

The growth of suckled mammary glands is affected by maternal nutrition. It has been shown that mammary growth is affected by both protein and energy intake and was maximized when primiparous sows of 200 kg body weight are provided with 55 g true ileal digestible lysine and 16.9 Mcal metabolizable energy daily during lactation (Kim *et al.*, 1999a) (Fig. 28.9). The National Research Council (1998) nutrient recommendations for swine suggest that a sow may require 49 g/day of lysine. Comparing with Kim *et al.* (1999a, b), the difference of 6 g/day lysine (49 vs. 55 g/day) could be accounted for by the need for mammary tissue growth during lactation, which was not taken into consideration when the National Research Council recommendations were established in 1998.

28.2.4 Litter size and mammary gland growth

With continuous genetic selection, a sow is more highly prolific than ever before. A sow in 2011 has three more piglets per litter than a sow in 1960 (National Agricultural Statistics Service, 2011). Increase in litter size directly increases the number of lactating mammary glands and thus a sow needs to have increased nutrient supply not just to produce more milk but also to support the growth of these mammary glands that are additionally needed to support the increased litter size. Milk yield is more than 50% greater when litter size increases from 6 to 12 (King *et al.*, 1989). This may result from an increased number of active mammary gland cells, a crucial component of milk production (Knight & Peaker, 1984; Knight *et al.*, 1984). Sows need an additional 1.0 g/day of lysine to account for mammary gland growth for each piglet added to a litter of 6 to 14 piglets (Kim *et al.*, 1999c).

Kim *et al.* (1999c) demonstrated that increase in litter size causes a decrease in the size of individual suckled mammary glands, indicating a potential decrease in milk production from each mammary gland. However, mammary glands of a sow with a large litter may be more efficient at producing milk because the reduction in individual piglet weight gain was only 73% of the decline in mammary gland growth rate observed in response to increased litter size (Kim *et al.*, 1999c). A 1-g increase in mammary protein in mammary glands was equivalent to an 80-g increase in litter weight gain. A 1-g increase in mammary DNA of total nursed mammary glands was equivalent to a 4.17-kg increase in litter weight. An increase in the size of mammary glands or the amount of mammary tissue protein resulted in a positive impact on piglet weight gain (Kim *et al.*, 2000). These results indicate that physiological or nutritional manipulations that result in increased mammary growth during lactation will coincide with enhanced litter weight gain and increased sow productivity.

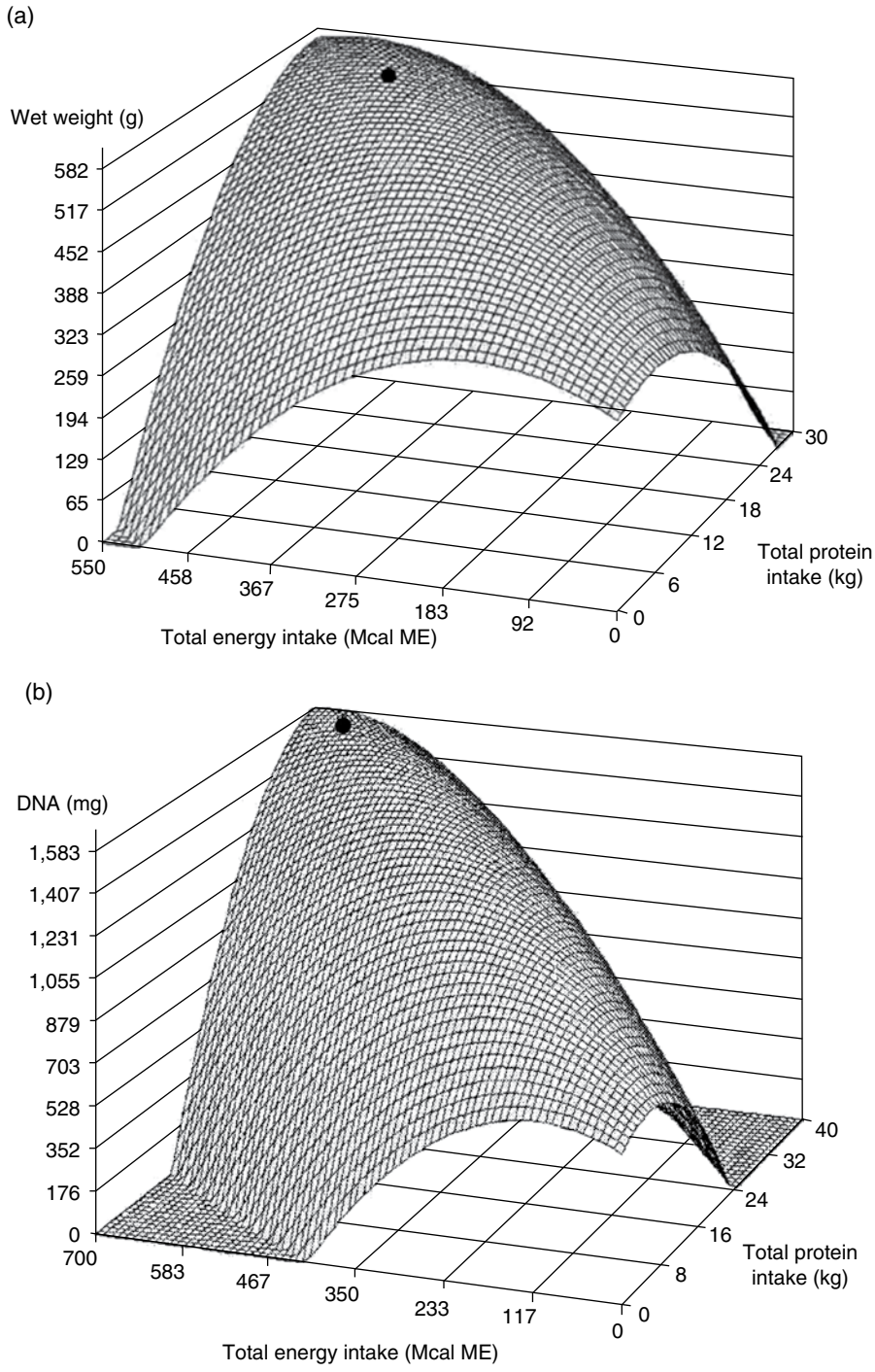


Figure 28.9. (a) Response surface plot of the weight of suckled individual mammary glands from sows fed different levels of energy and protein. Weights of suckled individual mammary glands are affected by maternal protein and energy intake during lactation. (b) Response surface plot of the DNA amount in suckled individual mammary glands from sows fed different levels of energy and protein. DNA content in suckled individual mammary glands is affected by maternal protein and energy intake during lactation. •, maximum stationary point. Day of lactation when the maximum stationary point occurred was 25.3. Adapted from Kim *et al*. (1999b), with permission of the American Society of Animal Science.

Table 28.1. Per cent composition of nutrients in porcine colostrum and milk.

			Holstein cow		Mare	
	Colostrum	**Milk**	**Colostrum**	**Milk**	**Colostrum**	**Milk**
Dry matter (total solids, %)	20–24	16–20	24	13	25	11
Protein (% DM)	50–67	25–30	59	24	76	24
Fat (% DM)	20–29	32–45	28	31	3	18
Lactose (% DM)	10–20	30–35	11	39	18	54
Ash (% DM)	1–4	4–5	5	6	31	4

DM, dry matter.

Sources: based on data from Schmidt (1971), Mateo *et al*. (2008, 2009), Lin *et al*. (2009), and Shen *et al*. (2011).

28.3 PORCINE COLOSTRUM AND MILK

At parturition, mammary glands start secreting colostrum to newborn piglets. A newborn piglet consumes colostrum equivalent to about 5–7% of its body weight during the first hours of nursing (Fraser & Rushen, 1992). On ingestion of colostrum, the gastrointestinal tract immediately undergoes rapid growth and differentiation (Smith & Jarvis, 1978). Nursing frequency progressively increases during the initial 24 hours after birth (Varley *et al*., 1987).

In a recent study, Lin *et al*. (2009) collected colostrum from 400 sows and measured nutrient concentrations. The composition of nutrients in colostrum is shown in Table 28.1 and is similar to the data presented by other researchers (Davis *et al*., 1994; Csapó *et al*., 1996; Kim & Wu, 2004). Colostrum contains 22% dry matter and, on a dry matter basis, 22% crude fat, 67% crude protein (such as casein), and 1.0% crude ash (such as minerals) leaving 10% as carbohydrates including lactose. This is similar to the nutrient composition of cow colostrum, except for ash percentage which is lower in sow colostrum than in cow colostrum (Table 28.1). Notably, the concentration of total amino acids (peptide-bound and free) in colostrum is 52.4% of dry matter, which is significantly higher than that in mature porcine milk. The most abundant amino acids (per cent of dry matter) are glutamate plus glutamine (7.8%), followed by lysine (6.6%) and proline (5.6%). Amino acids in colostrum are highly digestible and are well absorbed by newborn piglets, indicating that colostrum is a great source of nutrients and functional proteins for neonatal piglets. Digestibility of crude protein and dry matter in colostrum is 96.9 and 98.3%, respectively. Digestibility of total amino acids is 98.3%. Among all amino acids, digestibility of glutamate plus glutamine is the highest (98.8%), whereas that of glycine is lowest (97.9%). The nutrient composition of sow colostrum can be influenced by several factors, including variation among sows, varying disease status, genetic difference, dietary regimen, and body fat content (Klaver *et al*., 1981; Gooneratne *et al*., 1982; Zou *et al*., 1992; King *et al*., 1993; Jackson *et al*., 1995).

Immunoglobulin is an important protein for neonatal piglets with regard to passive immunity. In a piglet, transfer of immunoglobulin via colostrum is important because the placenta interferes with immunoglobulin transfer from mother to fetus. There are different isotypes of immunoglobulin. IgG is the major immunoglobulin found in porcine colostrum. IgG is transferred primarily from the maternal blood circulation to colostrum. IgA and IgM are mainly synthesized by B lymphocytes in mammary tissue. Porcine colostrum contains about 10–100 g/L IgG, 5–10 g/L IgA, and 2–3 g/L IgM (Butler, 1995; Mateo *et al*., 2008, 2009; Lin *et al*., 2009; Shen *et al*., 2011). However, not all immunoglobulins in colostrum are successfully transferred to a neonate. For example, only about 22–30% of IgG can be successfully transferred in its intact form during the first 3 days of life (Jensen *et al*., 2001; Lin *et al*., 2009).

After 12–24 hours of farrowing, milk let-down becomes periodic, i.e., 45–60 min intervals, and suckling is socially synchronized among littermates (Brooks & Burke, 1998). By 48–72 hours of farrowing, secretions from mammary alveoli change the composition as colostrum matures to milk, with significant reduction in IgG. Mature milk also has reduced dry matter and protein content compared with colostrum (Table 28.1). In mature milk IgA becomes the predominant immunoglobulin as IgG concentration decreases significantly. IgA effectively inhibits bacterial colonization of enterocytes and neutralizes viruses (Tizard, 1996). Nutrients in milk are considered to be highly digestible for nursing piglets. Mavromichalis *et al.* (2001) measured true ileal digestibility of amino acids in milk and demonstrated that the amino acids in sow milk are highly digestible (92%), even though the values are lower than those for colostrum (98%; Lin *et al*., 2009). Among amino acids, the digestibility of threonine, tryptophan, and

arginine in milk is lower than that of other amino acids (Mavromichalis *et al*., 2001).

The sow's ability to produce milk is one of the major limiting factors for piglet growth and survival (Kim *et al*., 2000). Normal healthy piglets possess the ability to ingest a greater amount of milk than that available from sows. Growth of piglets reared by artificial milk feeding was 30–80% greater than the piglets nursing sows (Boyd *et al*., 1995; Zijlstra *et al*., 1996). However, artificial milk feeding has not yet been accepted by the swine industry mainly due to high costs for dietary ingredients, facilities, and maintenance.

In a recent study, Voilqué *et al*. (2012) demonstrated that colostrum and milk composition is influenced by the production environments of sows. When sows were kept in heat stress environment, protein content (46% of dry matter) in colostrum decreased compared with sows kept in moderate temperature environment (53% of dry matter). However, the composition of fat and lactose in colostrum was not affected by altering the temperature environment. Mature milk composition was not affected by different temperature environments. Age of sows can be related to the composition of colostrum and milk. Voilqué *et al*. (2012) demonstrated that an increase in parity from two to six increased fat content in colostrum, whereas other components in colostrum and milk were not affected. However, body weight of sows did not affect the composition of colostrum and milk. Litter size can also affect the composition of colostrum and milk. Voilqué *et al*. (2012) observed increases in lactose content (from 20 to 31% of dry matter) and protein content (from 23.0 to 28.8% of dry matter) as litter size increased from 8 to 14 piglets at birth.

28.4 DIETARY MANIPULATIONS THAT AFFECT MILK PRODUCTION AND COMPOSITION

Adequate feeding during lactation to meet the nutrient requirements of sows is important to achieve maximal milk production. Kim *et al*. (1999a) have demonstrated that maternal nutrition during lactation affects milk production. Increasing both energy and lysine intakes enhances growth of the mammary gland and the number of mammary epithelial cells.

Sows respond differently to dietary regimens depending on their parity or age. The nutrient requirements of lactating sows vary with parity (National Research Council, 1998); furthermore, sows require different quality of protein with varying ideal amino acid profile according to parity (Kim *et al*., 2001b). It is rather common that sows lose body weight during lactation, mobilizing their body tissues to replace the deficit of nutrients in order to support the growth of mammary tissue and milk production (Kim & Easter, 2003). When sows are fed adequately, sows seem to use different routes for metabolizing nutrients (Pluske *et al*., 1998). Primiparous sows seem to partition extra energy into body growth rather than into milk production, whereas multiparous sows use it for milk production.

Table 28.2. Effect of supplemental fat on litter performance, milk production, and composition.

	Control	Tallow	SE
Number of sows	17	17	—
Litter size at day 21	9.60	9.21	0.25
Litter weight (kg)			
Day 0	15.5	15.0	0.4
Day 21	58.8	57.3	1.7
Day 28	75.2	74.1	2.1
Milk production (kg/day)	9.25	8.97	0.87
Milk composition			
Solids (%)	19.5[a]	21.2[b]	0.2
Protein (%)	5.3	5.1	0.1
Fat (%)	7.7[a]	9.6[b]	0.2
Ash (%)	0.79[a]	0.83[b]	0.01
Gross energy (Mcal/kg)	1.19[a]	1.37[b]	0.02

Within a row, means lacking common superscripts differ ($P < 0.05$).

Source: adapted from Tilton *et al*. (1999), with permission of the American Society of Animal Science.

Supplementation with functional nutrients can modify milk composition. Dietary conjugated linoleic acid in the diet reduced the fat content of sow milk without affecting piglet growth and the energy demands of lactation (Harrell *et al*., 2002). Tilton *et al*. (1999) showed that sows consuming diets supplemented with tallow produced milk with increased composition of solids, fat, ash, and gross energy (Table 28.2). Dietary supplementation with yeast and yeast metabolites seems to affect milk composition and production. Feeding lactating sows with live yeast or yeast metabolites increased the proteins in sow milk and enhanced litter weight gain (Kim *et al*., 2008, 2010a; Shen *et al*., 2011).

Catabolic condition of sows during lactation reduces blood flow adversely, affecting nutrient availability to mammary glands for milk production (Kim & Wu, 2009). Arginine enhances blood flow (Kim *et al*., 2010b) when it is metabolized to citrulline, producing nitric oxide in endothelial cells lining the blood vessel. Dietary supplementation of a lactation diet with arginine increased litter weight gain, indicating increased milk production (Mateo *et al*., 2008). Similar effects have been shown when nitric oxide has been introduced by dietary supplementation with nitric oxide donors to sows during lactation (Kim & Wu, 2009). Adequate use of selected functional nutrients will

therefore help the growth and health of piglets by enhancing milk production and composition.

28.5 SOW MILK IN HUMAN NUTRITION RESEARCH

Pigs have been used widely as a model for human nutrition and medical research due to their similarity with humans with regard to digestion and metabolism as well as in the structure of vital organs and circulatory systems (Kearns *et al*., 1986; Peggins *et al*., 1986; Sharma *et al*., 1996; Park, 2006). Research on nutrition and reproductive performance related to milk production, milk composition, and neonatal growth in sows has been of great value to studies on human nutrition and mother–infant relations (Dvorchik, 1981; Juchau, 1990; Mateo *et al*., 2007, 2009; Kim & Wu, 2009). Sows have also been used for genomic research. Transgenic sows created by inserting bovine genes for α-lactalbumin produced more α-lactalbumin in milk and this resulted in enhanced growth of nursing piglets (Noble *et al*., 2002).

28.6 SUMMARY

A domestic pig is highly prolific, typically giving birth to 10–16 piglets. Growth and health of nursing piglets is closely related to the capability of the mammary glands because milk synthesis occurs in mammary epithelial cells and this determines the quantity and quality of milk. Mammary glands undergo physiological and morphological changes at the onset of pregnancy and grow substantially during gestation. Mammary gland growth in pregnant gilts shows that mammary parenchymal tissues increase 24-fold during gestation. Compositional changes occur in mammary glands during gestation and lactation. The percentage of dry matter in fresh mammary parenchymal tissue decreases. The percentage of protein in dried mammary parenchymal tissue increases whereas the percentage of fat in dried mammary parenchymal tissue decreases from breeding to weaning. These compositional changes are caused by structural changes as a greater proportion of the tissue is composed of adipocytes and connective tissues at early gestation but is replaced by alveoli as gestation progresses. Mammary tissue growth during lactation requires 6 g/day true ileal digestible lysine. Mammary growth is maximized when a primiparous sow weighing 200 kg consumes 55 g true ileal digestible lysine and 16.9 Mcal metabolizable energy daily during lactation. At parturition, mammary glands start secreting colostrum to newborn piglets. This contains 20–24% dry matter and, on a dry matter basis, 20–29% crude fat, 50–67% crude protein, 10–20% lactose, and 1–4% crude ash. The concentration of total amino acids (peptide-bound and free) in colostrum is 52.4% of dry matter, which is significantly higher than that in mature porcine milk. Digestibility of total amino acids is 98.3%. The nutrient composition of sow colostrum can be influenced by several factors, including variation among sows, varying disease status, genetic difference, dietary regimen, and body fat content. Porcine colostrum contains about 10–100 g/L IgG, 5–10 g/L IgA, and 2–3 g/L IgM. However, not all immunoglobulin in colostrum is successfully transferred to the neonate: only about 22–30% of IgG can be successfully transferred in its intact form during the first 3 days of life. Colostral protein content is decreased under a heat stress environment and as a sow ages, whereas it is increased as litter size increases. Maternal nutrition influences production and composition of colostrum and milk and thus formulation of a diet should consider sow requirements based on parity and age. Research on nutrition and reproductive performance related to milk production, milk composition, and neonatal growth in sows has been of great value to studies on human nutrition and mother–infant relations.

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29
Yak Milk

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29.1 INTRODUCTION

The yak (*Poephagus grunniens* or *Bos grunniens*) thrives in conditions of extreme harshness and deprivation while providing a livelihood for people. The yak lives predominantly on the Qinghai-Tibetan Plateau, which comprises alpine and subalpine regions at altitudes of 2000–5000 m in the western part of China. The characteristics of these regions are extreme cold, high altitude with low oxygen content of the air, and high solar radiation with short growing seasons for grazing herbages as well as a variety of other plants (Wiener *et al*., 2003). According to the Chinese provincial annals of livestock breeds, there are 12 officially recognized breeds of domestic yak in China: the Jiulong yak and Maiwa yak in Sichuan province, Tianzhu White yak and Gannan yak in Gansu province, Pali yak, Jiali ("Alpine") yak and Sibu yak in Tibet, Huanhu yak and Plateau yak in Qinghai province, Bazhou yak in Xinjiang and Zhongdian yak in Yunnan province, and one other, the "Long-hair-forehead yak" in Qinghai province. These 12 yak breeds belong to two main types, the Qinghai-Tibet Plateau type (Plateau or Grassland type) and the Henduan Alpine type (Alpine or Valley type) (Wiener *et al*., 2003). Yak herds are also found in the Republic of Mongolia, Nepal, Kirgisia, India (Table 29.1) and even in North America, where about 500 head were kept on different ranches in 1992 and an International Yak Association and Registry was organized in 1993 (Kirkham, 1992). Yak are also often crossbred with other cattle and Zebu breeds for improved milk and meat production, but the males are sterile.

There are differences in chemical characteristics between yak and cow milk. Yak milk is predominantly produced by seasonal breeding. Its composition varies with seasonal grass growth and climatic change, as does milk production. The highest content comprises solids, lactose, protein and amino acids and occur in the mid-lactation period, but the fat content increases continuously into late lactation (Zhang & Pu, 1986; Ji *et al*., 2000). In contrast, the bulk milk from dairy cow herds varies little with the seasons because of year-round breeding; therefore the composition of cow milk shows minimal changes throughout the year. Milk is a complex colloidal dispersion containing fat globules, casein micelles, and whey proteins in an aqueous solution of lactose, minerals, and a few other minor compounds. Its physical and chemical properties depend on intrinsic compositional and structural factors and extrinsic factors such as temperature and post-milking treatments. This chapter focuses on basic information for yak milk. Most data come from the authors' research group. Some data have not yet been published in journals.

29.2 BASIC COMPOSITION

Yak milk is a naturally concentrated milk because of its high content of fat (5.5–7.5%), protein (4.0–5.9%), and lactose (4.0–5.9%) during the main lactation period (Zhong & Yu, 1996; Yu *et al*., 2005). However, milk production is limited by low volume and seasonal cyclicity, with a yak producing around 150–500 kg of fresh milk per lactation depending on breed, age, parity and body

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Table 29.1. Milk composition (%) of different yak breeds.

Yak breeds (country)	Milk solids	Fat	Protein	Lactose	Ash
Tianzhu White yak (China)	16.31–18.38	5.64–5.77	4.71–6.53	5.02–5.31	0.77–0.87
Jiulong (China)	17.28–17.76	6.85–7.23	4.85–4.88	4.71–4.83	0.79–0.83
Maiwa (China)	17.51	6.34	4.92	5.43	0.82
Inner Mongolia (China)	17.78	6.79	5.03	5.10	0.86
Lulang (China)	18.00	6.92	4.95	4.77	0.79
Songduo (China)	18.36	7.13	5.02	5.09	0.81
Milashan (China)	19.00	7.38	5.20	5.15	0.83
Jiali (China)	16.31	6.75	5.0275	3.56	0.95
Pali (China)	16.32	5.95	5.73	3.77	—
Sibu (China)	17.11	7.50	5.27	3.49	—
Kirghizia	17.35	6.6	6.32	4.62	0.87
Nepal	17.40	6.50	5.40	4.60	0.90
India	17.93	6.45	5.94	4.68	0.87

Source: based on data from Li *et al*. (2009).

Table 29.2. Basic chemical composition (%) of Maiwa yak milk from different seasons.

	Maiwa yak milk[1]					
	May	September	October	December	Cow milk[2]	Buffalo milk[3]
Fat	5.11 ± 1.48	6.31 ± 0.25	6.77 ± 0.96	6.99 ± 1.20	4.5	7.59
Total protein	5.25 ± 0.35	5.18 ± 0.10	4.96 ± 0.50	4.22 ± 0.17	2.9	4.86
Lactose	5.53 ± 0.33	5.12 ± 0.02	4.95 ± 0.81	4.40 ± 0.55	4.1	4.74
Ash	0.79 ± 0.03	0.85 ± 0.01	0.81 ± 0.06	0.72 ± 0.05	0.8	0.85
Total solids	16.19 ± 1.47	17.46 ± 0.14	17.35 ± 1.28	16.49 ± 1.49	12.7	18.44

Sources: [1]based on data from Li *et al*. (2011); [2]based on data from Fox & McSweeney (2003); [3]reproduced from Han *et al*. (2007), with permission of Elsevier.

condition, pasture growth, pasture quality, raising areas, milking time, milking methods, and other environmental factors (Wiener *et al*., 2003).

Yak milk is of particular interest due to its specific composition, which has led to it being considered a high-quality raw material for manufacturing food for infants and the elderly, and for certain sectors of the population with particular needs. The basic composition of yak milk from different breeds and locations is listed in Table 29.1.

The Maiwa yak outnumbers all the other kinds of yak. Maiwa yak milk composition from different seasons is listed in Table 29.2, where it is compared with cow and buffalo milk. The content of fat and total protein in yak colostrum is higher than in cow and goat colostrum (Table 29.3). The high concentration of protein in colostrum may be partly due to the high IgG level in this fraction (Moreno-Indias *et al*., 2012). Thus, yak colostrum is richer in immune factors than cow and goat colostrum.

The high content of fat, protein, lactose and total solids of yak milk is comparable with that of cow milk (Fox & McSweeney, 2003), but is in fact more similar to buffalo milk (Han *et al*., 2007). Because the yak is grazed under uncontrolled environmental conditions, milk composition varies with seasonal grass growth and climate changes. On the Qinghai-Tibet Plateau, the average annual air temperature is generally bellow 0°C, while the average temperature in January drops below –10°C. The average temperature in the warmest month (July) does not exceed 13°C. A year is divided into two seasons, cold and warm. The cold season is from October to April, and the warm season from May to September. The calving season of Maiwa yak is usually in May to June. In the warm season, there is plenty of green grass to feed the yak. The cold pasture on which yak graze comprises predominantly short grass and rough grazing with sedges and shrubby plants (Wiener *et al*., 2003). In the cold season (October and December), yak milk has higher

Table 29.3. Basic composition (%) of yak colostrum and other mammalian colostrums.

	Yak colostrum[1]	Cow colostrum[2]	Goat colostrum[3]
Fat	14.00	5.74	8.7
Total protein	16.14	6.54	10.4
Lactose	1.86	3.49	2.1
Ash	1.01	0.90	1.57
Total solids	33.01	16.98	22.77

Sources: [1]reproduced from Park & Haenlein (2006), with permission of Blackwell Publishing; [2]based on data from Lu *et al*. (2001); [3]based on data from Moreno-Indias *et al*. (2012).

Table 29.4. Physical properties of yak milk and milk of other mammals.

	Maiwa[1]	Zhongdian[1]	Gannan[1]	Bovine milk[2]	Goat milk[2]	Sheep milk[2]
Specific gravity (d^{20})	1.03 ± 0.01	1.03 ± 0.01	1.03 ± 0.01	1.02–1.04	1.02–1.04	1.03–1.04
Density, ρ^{20} (kg/m^3) × 10^3	1.03 ± 0.01	1.03 ± 0.01	1.03 ± 0.01	1.03	—	—
Refractive index	1.35 ± 0.01	1.35 ± 0.01	1.35 ± 0.01	1.34–1.35	1.45 ± 0.39	1.35
Titratable acidity (°T)	20.83 ± 0.29	20.53 ± 0.12	21.00 ± 0.15	15.00–18.00	14.00–23.00	22.00–25.00
pH	6.71 ± 0.01	6.73 ± 0.01	6.78 ± 0.01	6.65–6.71	6.50–6.80	6.51–6.85
Surface tension (mN/m)	43.79 ± 0.16	42.93 ± 0.47	47.94 ± 0.46	42.3–52.1	52.00	44.94–48.70
Freezing point (°C)	–0.50 ± 0.01	–0.50 ± 0.01	–0.50 ± 0.01	–0.53–0.57	–0.54–0.58	–0.57
Boiling point (°C)	104.00 ± 0.01	102.00 ± 0.01	103.00 ± 0.01	100.00–101.00	—	—
Electrical conductivity ($\times 10^4$ μS/cm)	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.40–0.55	0.43–1.39	0.38
Viscosity (mPa·s)	1.95 ± 0.01	1.92 ± 0.01	1.97 ± 0.01	1.50–2.00	2.12	2.86–3.93
Specific heat (J/g·°C)	3.95 ± 0.04	3.91 ± 0.04	4.11 ± 0.06	3.84	—	—
Casein micelle size (nm)	173.11 ± 14.36	176.33 ± 11.59	186.97 ± 2.57	180	260	193

Sources: [1]courtesy of H. Li. Reproduced from Li (2011), with permission; [2]reproduced from Park *et al*. (2007), with permission of Elsevier.

fat content and lower protein and lactose content than milk in the warm season (Table 29.2). With the maturing growth of forage (from August to December), the protein content in the sward declines from 115 g/kg DM (young grass) to 33 g/kg DM (mature grass), and crude fiber in the sward increases correspondingly (Wiener *et al*., 2003). The increased crude fiber offers more acetic and butyric acids (sources of fatty acids) for the mammary gland to synthesize more fat (Wiener *et al*., 2003). The fat content in sheep milk undergoes similar seasonal changes from February to August (7.6% to 6.6%) (Jaeggi *et al*., 2005).

The higher content of lactose in yak milk benefits infants. Lactose in the human distal intestine can help combat gastrointestinal disturbances resulting from undesirable putrefactive bacteria by promoting the growth of certain beneficial lactic acid-producing bacteria (Miller *et al*., 2000).

The ash content of yak milk is similar to that of cow milk and is constant throughout the cold and warm seasons. Jiang *et al*. (1993) analyzed the ash content of Maiwa yak milk from June to September (0.82 ± 0.06%) and found that there was no significant change with the seasons.

29.3 PHYSICAL CHARACTERISTICS

Information on the physical properties of milk is very important, since such parameters can influence the design and operation of dairy processing equipment, can be used to determine the concentration of specific components in milk, or can be used to assess the extent of biochemical changes in the milk during processing (Fox & McSweeney, 1998). Although there is limited information about yak milk, some physical parameters are different from those that characterize the milk of cows, goats, and sheep (Table 29.4); however, these parameters are similar between different yak breeds.

Measurement of the density of whole milk is one way of checking for extraneous water contamination, and it provides a rapid means of indirectly determining total solids (McSweeney & Fox, 2009). Refractive index measurements can be used satisfactorily to estimate the

Table 29.5. Physical properties of Maiwa yak milk from different seasons.

	Warm season		Cold season	
	May	**September**	**October**	**December**
Specific gravity (d^{20})	1.0325 ± 0.01[a]	1.0331 ± 0.01[a]	1.0283 ± 0.01[a]	1.0330 ± 0.01[a]
Density, ρ^{20} (kg/m^3) × 10^3	1.032 ± 0.01[a]	1.032 ± 0.01[a]	1.032 ± 0.01[a]	1.032 ± 0.01[a]
Refractive index	1.3514 ± 0.001[a]	1.3541 ± 0.001[a]	1.3528 ± 0.001[a]	1.3530 ± 0.001[a]
Titratable acidity (°T)	21.00 ± 0.15[a]	23.47 ± 0.31[c]	23.03 ± 0.49[b]	23.42 ± 0.52[b]
pH	6.56 ± 0.06[a]	6.55 ± 0.01[a]	6.56 ± 0.01[a]	6.53 ± 0.03[a]
Surface tension (mN/m)	40.60 ± 5.13[a]	43.27 ± 0.21[a]	42.93 ± 4.37[a]	41.47 ± 3.43[a]
Freezing point (°C)	–0.5 ± 0.01[a]	–0.5 ± 0.01[a]	–0.5 ± 0.01[a]	–0.5 ± 0.01[a]
Boiling point (°C)	102.35 ± 1.33[a]	104 ± 1.24[a]	102.50 ± 1.00[a]	101.77 ± 1.32[a]
Electrical conductivity (×10^4 μS/cm)	0.35 ± 0.01[b]	0.32 ± 0.01[a]	0.31 ± 0.02[a]	0.32 ± 0.02[c]
Viscosity (mPa·s)	2.08 ± 0.25[a]	2.12 ± 0.03[a]	2.57 ± 0.48[b]	2.31 ± 0.35[b]
Specific heat (J/g·°C)	3.85 ± 0.01[b]	4.10 ± 0.03[d]	3.40 ± 0.01[a]	3.91 ± 0.01[c]
Casein micelle size (nm)	167.03 ± 22.84	178.47 ± 8.01	—	207.17 ± 3.97

Within a row, means lacking common lower case superscripts differ ($P < 0.05$).
Source: courtesy of H. Li. Reproduced from Li (2011), with permission.

solids-not-fat content of milk and condensed milk. Generally, there is a linear relationship between solids content (based on weight per unit volume) and refractive index, but this relation varies between different lots of milk, owing mainly to variations in the lactose/protein ratio (McSweeney & Fox, 2009). The higher titratable acidity of yak milk shows that it has high viscosity and protein content, because titratable acidity of milk is related to the content of protein and buffer components of milk (McSweeney & Fox, 2009). The surface tension of milk is a fundamental physical property, and it affects fractionation, concentration, and drying processes. The surface tension of milk is influenced by surface-active components (casein micelles, phospholipids, whey proteins and fatty acids) and fat content that can readily adsorb at an air–water interface and reduce surface tension. The surface tension of yak milk is similar to that of cow milk, indicating that yak milk and cow milk have similar physical properties, such as the stability of foams, emulsions and films (McSweeney & Fox, 2009). Higher protein and fat content result in the higher viscosity of yak milk. The viscosity of milk is most affected by protein and relatively by fat, but lactose, the major low-molecular-weight milk constituent, and even whey proteins influence viscosity to a relatively small extent (Sherbon, 1999).

Some physical properties of Maiwa yak milk from cold and warm seasons are shown in Table 29.5. Except for viscosity, titratable acidity, electrical conductivity and specific heat, other parameters do not exhibit significant differences between seasons. Usually, milk density is not affected by environmental conditions, stage of lactation, lactation number, or nutritional level of the animal, aside from the effects of these parameters on milk composition (Sherbon, 1999; Park *et al*., 2007). The viscosity of Maiwa yak milk in the cold season is higher than that in the warm season, due to the higher fat content during the cold season. In the dairy industry, measurements of titratable acidity can follow bacterial actions or viscosity and assess the aggregation of protein micelles or fat globules. A high initial acidity (in the absence of lactic acid development) of yak milk suggests that the milk is rich in proteins and other indigenous buffering constituents (McSweeney & Fox, 2009).

29.4 PROTEINS

29.4.1 Nitrogen distribution

The nitrogen-containing portions of milk can be divided into three broad fractions, including casein (CN), whey protein (WPN), and non-protein nitrogen (NPN). The content of NPN and the ratio of NPN to total nitrogen (TN) in yak milk is different between three yak breeds (Table 29.6). All the components of NPN are present in blood, from where they are transferred into milk. The technological and nutritional significance of NPN is not known but the amino acids are important for the nutrition of starter microorganisms, especially weakly proteolytic strains (Fox & McSweeney, 1998). The NPN fraction is composed of urea and other low-molecular-weight compounds such as creatine and creatinine. The single largest contributor to the NPN fraction of milk is urea. Urea equilibrates in body water, and blood urea is the primary source of milk urea. The urea in milk can be derived from at least two sources:

Table 29.6. Nitrogen distribution (%) in milk of different yak breeds.

	Zhongdian[1] ($N=56$)	Ganan[1] ($N=48$)	Maiwa[1] ($N=114$)	Cow milk[2]
TN	0.68 ± 0.02	0.84 ± 0.06	0.79 ± 0.04	0.36–0.69
NPN	0.07 ± 0.11	0.03 ± 0.02	0.04 ± 0.01	0.023–0.042(0.03)
NPN/TN	10.29	3.58	5.06	~5
WPN	0.13 ± 0.52	0.17 ± 0.04	0.15 ± 0.01	
WPN/TN	19.12	20.23	18.99	~17
CN	0.48 ± 0.17	0.64 ± 0.06	0.60 ± 0.03	
CN/TN	70.59	76.19	75.95	78
WPN/CN	27.08	26.41	25	22

TN, total nitrogen; NPN, non-protein nitrogen; WPN, whey protein nitrogen; CN, casein.

Sources: [1]based on data courtesy of H. Li. Reproduced from Li (2011), with permission; [2]based on data from Fox & McSweeney (2003).

Table 29.7. Nitrogen distribution (%) in Maiwa yak milk from different seasons.

	Warm season		Cold season	
	May	September	October	December
TN	0.82 ± 0.35^{b}	0.81 ± 0.10^{b}	0.78 ± 0.50^{b}	0.66 ± 0.16^{a}
NPN	0.05 ± 0.06^{a}	0.04 ± 0.01^{a}	0.04 ± 0.13^{a}	0.3 ± 0.05^{a}
NPN/TN	5.52	5.41	5.64	5.21
WPN	0.15 ± 0.12^{a}	0.16 ± 0.16^{a}	0.17 ± 0.28^{a}	0.15 ± 0.10^{a}
WPN/TN	19.05	19.30	21.04	21.09
CN	0.62 ± 0.33^{b}	0.61 ± 0.19^{b}	0.57 ± 0.61^{b}	0.48 ± 0.25^{a}
CN/TN	75.43	75.29	73.80	71.80
WPN/CN	25.24	25.64	28.95	29.37

TN, total nitrogen; NPN, non-protein nitrogen; WPN, whey protein nitrogen; CN, casein.
Within a row, means lacking common lower case superscripts differ ($P<0.05$).
Source: courtesy of H. Li. Reproduced from Li (2011), with permission.

the end product of digestion and amino acid catabolism. Blood urea nitrogen is positively associated with intakes of rumen-degradable and -undegradable protein and negatively associated with intake of net energy (DePeters & Ferguson, 1992). NPN constitutes only 5–6% of TN in cow milk, which is relatively minor compared with the CN and WPN fractions of milk nitrogen, but it is important to cow and human metabolism (DePeters & Ferguson, 1992). The NPN content of milk is less variable among breeds, but the range within a given breed is considerable. The level of NPN in freshly drawn milk is fairly constant but it does increase on aging, especially if significant growth of psychrophilic bacteria, which may be strongly proteolytic, occurs (Fox & McSweeney, 1998). The NPN distribution in TN of yak milk ranges from 3.6 to 10.3% (Table 29.6), which is quite variable and deserving of further research.

Yak WPN accounts for 19–20% of TN. The ratio of WPN to TN in yak milk is higher than that in cow milk (DePeters & Ferguson, 1992), but is similar to that in sheep milk (Park *et al*., 2007). Whey proteins are an excellent source of dietary nitrogen and essential amino acids. Because of the classical nutritional and functional attributes of whey proteins, whey proteins and their associated peptides display significant potential as functional food ingredients. In particular, whey proteins/peptides have potentially beneficial human health effects, such as hypotensive, anticancer, immunomodulatory, opioid agonist and antagonist, mineral-binding, antimicrobial, gut health enhancing, hypocholesterolemic, insulinotrophic and psychomodulatory activities.

Variations in the nitrogen distribution of yak milk proteins from warm and cold seasons are shown in Table 29.7. The TN of yak milk during the warm season is higher than that for the cold season. The protein content is affected by several factors, including breed, environmental temperature, diseases, stage of lactation, parity, and nutrition

(DePeters & Ferguson, 1992). In general, high environmental temperatures reduce the total protein content of milk (Fegan, 1979). The protein concentration of cow milk is higher during winter than during summer (DePeters & Ferguson, 1992). However, several studies have demonstrated that energy intake (except fats and oils) and milk protein percentage are positively correlated (Emery, 1978; Fegan, 1979; DePeters & Palmquist, 1990). On the Qinghai-Tibet Plateau, grass and herbs cannot survive a very cold winter. For natural-grazing yak, malnutrition results in lower TN in winter. Similarly, Walley and O'Connor (1980) demonstrated that the total protein content of milk was lowest when the energy intake of the cow was retarded. The NPN concentration of yak milk is approximately 0.22–0.28 g/dL of milk and is similar to that of cow milk (DePeters & Ferguson, 1992). The NPN content of milk is less variable among breeds, and changes in milk NPN content with environmental temperature are similar in pattern to protein content (DePeters & Ferguson, 1992).

Variation in CN parallels that of WPN in yak milk. The highest level of CN is found in the warm season, which is consistent with the maximum total protein content (Regester & Smithers, 1991; Guo *et al*., 2007). The ratio of CN to TN ranges from 72 to 75% from May to December, which is lower than in cow milk. For yak, although there are no papers presenting the changes in the percentage of CN to TN with season, the variation patterns of CN and CN/TN are consistent with those found in North American commingled goat milk (Guo *et al*., 2007). The nitrogen distribution of yak milk shows significant variability among warm and cold seasons. Although limited data demonstrated this result, this variability follows a seasonal pattern that might be useful in deciding the end use of yak milk.

29.4.2 Protein composition

As in most other mammalian milks, proteins of yak milk mainly consist of the four individual caseins (α_{s1}, α_{s2}, β and κ) and the major whey proteins (α-lactalbumin, β-lactoglobulin, serum albumin, lactoferrin, and immunoglobulins) (Sheng *et al*., 2008). A reversed-phase high-performance liquid chromatography profile of yak milk proteins is presented in Fig. 29.1. As for cow κ-CN, yak κ-CN chromatography shows three or four peaks for this casein, indicating not only the genetic variation described for the gene *CSN3* by Prinzenberg *et al*. (2008) but also several likely forms of glycosylation (Swaisgood, 1992). Because cow α_{s1}-CN and α_{s2}-CN are not available as single proteins, the corresponding values for homologous yak caseins were calculated from the α_s-CN determination by applying the 4 : 1 proportion known for bovine milk (Bonizzi *et al*., 2009). With regard to β-CN, some individual Maiwa yak milk samples show two β-casein peaks. Such results, which

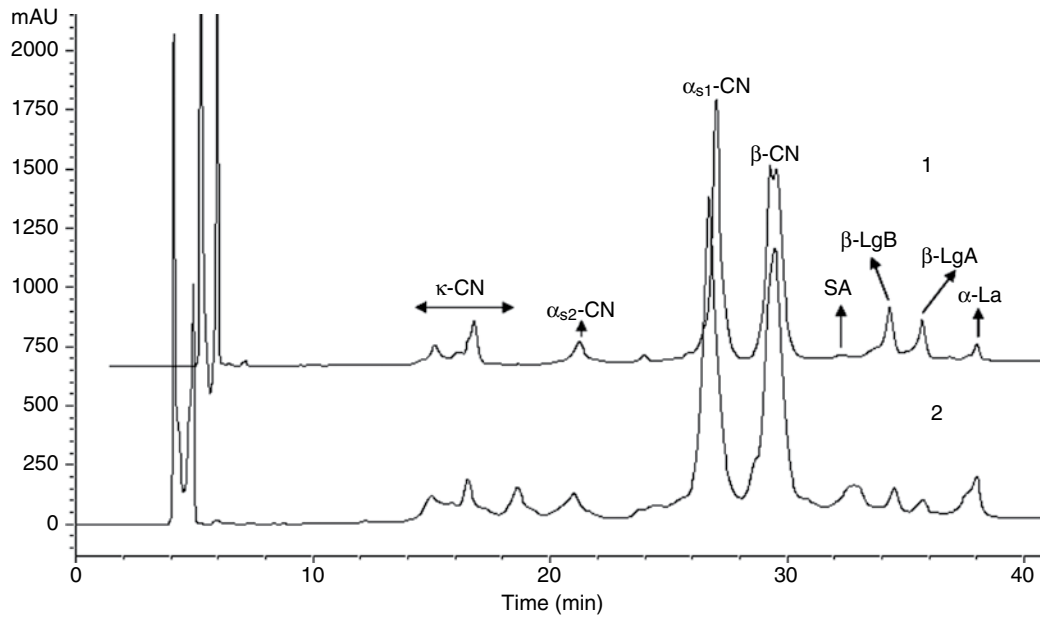


Figure 29.1. Chromatograms of bovine milk proteins and skim yak milk. CN, casein; α-La, α-lactalbumin; β-Lg, β-lactoglobulin.

Table 29.8. Mean concentration of casein (CN) fractions in Maiwa yak, bovine and human milk.

	Casein (g/L)	κ-Casein (g/L)	α_{s1}-Casein (g/L)	α_{s2}-Casein (g/L)	β-Casein (g/L)	κ-CN/CN (%)	α_{s1}-CN/CN (%)	α_{s2}-CN/CN (%)	α_s-CN/CN (%)	β-CN/CN (%)
Individual Maiwa yak milk[1] *(N = 24)*										
Mean	40.21	5.98	11.23	4.8	18.2	14.87	27.93	11.94	39.87	45.26
SD	3.17	0.95	0.95	0.71	1.52	2.35	2.37	1.78	3.78	3.77
Min.	34.28	4.94	9.25	3.62	14.95	12.29	23.00	9.00	37.54	37.18
Max.	45.79	8.54	13.07	6.48	20.57	21.24	32.50	16.12	42.69	51.16
Bovine milk[2]										
Mean	26.80	3.40	10.32	2.68	9.60	12.70	38.51	10.00	48.51	35.82
Human milk[3]										
Mean	3.70	0.87	0.43		2.40	23.50	11.75			64.75

Sources: [1] based on data from Li *et al*. (2010); [2] based on data from Walstra *et al*. (1999); [3] reproduced from Malacarne *et al*. (2002), with permission of Elsevier.

likely indicate polymorphism of this individual casein, disagree with those of Mao *et al*. (2004), who found it monomorphic, but agree with the hypothesis of Prinzenberg *et al*. (2008). As for most of the mammalian milks, yak milk caseins show a complex qualitative genetic polymorphism.

Total casein content of yak milk (40.2 g/L on average; Li *et al*., 2010) is 1.5 times the concentration found in cow milk (Walstra *et al*., 1999) and 11 times that of human milk (Table 29.8) (Malacarne *et al*., 2002). The high proportion of β-CN (more than 45% on average and ranging from 37.18 to 51.16%) results in a smooth and soft coagulum in the human stomach, which is easily digested by the enzymes of the intestinal tract. This perhaps explains why yak milk is usually given, after dilution, to babies by Tibetan nomads to complement breast milk (Wiener *et al*., 2003). On the other hand, it would be interesting to determine the sequence of yak β-CN to elucidate whether the bioactive peptides β-CN 1–25, 60–65, 177–183, etc. are present and have the same physiological abilities as their homologous sequences in human and cow β-CN (Léonil *et al*., 2001). Also, the high proportion of β-CN and consequently the lower proportion of α_s-CN (about 40% for bovine milk), together with a small increase in the κ-CN proportion (15% instead of 12% for bovine milk), will influence the cheesemaking ability of yak milk (renneting time, final firmness of the curd, whey drainage, etc).

The few data available in the literature concerning whey proteins in yak milk show that the proportion of β-lactoglobulin in total protein appears to be in the same range as in cow milk. On average, there was a higher proportion of serum albumin in yak milk compared with cow milk, but the individual variations were high. The value found for α-lactalbumin in yak milk is puzzling because it is not consistent with the usual relationship between lactose and α-lactalbumin content in mammalian milks (Fox & McSweeney, 2003). Indeed, α-lactalbumin is half of the lactose synthetase enzyme (Fox & McSweeney, 2003) and its content increases with lactose concentration.

29.4.3 Minor proteins

Milk contains numerous minor proteins with physiological properties targeted at providing immunoprotective, growth and antimicrobial factors to the neonate, as distinct from the nutritionally more significant major proteins. Many of these minor bioactive proteins are found in the serum fraction of mammalian milks, and are generally present at elevated levels in colostrum, which reflects their importance to early neonatal health. The minor proteins include immunoglobulins, lactoferrin, transforming growth factor (TGF)-β, milk fat globule membrane protein, and proteose peptones.

Lactoferrin (formerly known as lactotransferrin) is a glycoprotein and a member of the transferrin family, thus belonging to those proteins capable of binding and transferring Fe^{3+} ions. Lactoferrin has been the focus of intense research of late. Because of its unique antimicrobial, immunomodulatory, and even antineoplastic properties, lactoferrin seems to have great potential in practical medicine. As in other mammalian milks, the lactoferrin concentrations of Maiwa yak milk exhibit large variations depending on the season (Table 29.9). Nevertheless, much research still needs to be carried out in order to obtain a better understanding of its activity and interactions, and to enable full and safe utilization of this glycoprotein.

The physiological function of immunoglobulins is to provide various types of immunity in the body. The major effects of milk and colostral immunoglobulins include preventing microbes from attaching to epithelia, neutralizing viruses and toxins, and augmenting phagocytosis by leukocytes in milk. The major immunoglobulin classes in bovine and human milk are IgA, IgG, and IgM. The concentration

Table 29.9. Lactoferrin content of different animal milks in different months.

	Lactoferrin (μg/mL)	Month	Method	Reference
Maiwa yak	40	June	SDS-PAGE	Zheng (1992)
	376	August	SDS-PAGE	Jin *et al*. (2006)
	670	October	SDS-PAGE	Sheng *et al*. (2008)
	0.50–11.92	November	ELISA	Cheng (2011)
Camel milk	20–2100	Stage of lactation: beginning, middle and late	ELISA	Ahmad *et al*. (2007)
Goat milk	98–149	April to November	HPLC	Dračková *et al*. (2009)
Bovine milk	20–200			Park *et al*. (2007)
Human milk	<2000			Park *et al*. (2007)

SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay.

Table 29.10. IgG content of yak and other mammalian milks.

	IgG (mg/mL)	Month	Method	Reference
Maiwa yak	3.18–4.82	June	SDS-PAGE	Zheng (1992)
	0.554	August	SDS-PAGE	Jin *et al*. (2006)
	0.186	October	SDS-PAGE	Sheng *et al*. (2008)
	0.058–0.358	November	ELISA	Cheng (2011)
	0.652–1.071	July, September, November	SDS-PAGE	Zheng *et al*. (2002)
Jiulong yak	0.530–0.704	July, September, November	SDS-PAGE	Zheng *et al*. (2002)
Cow	0.59			Park *et al*. (2007)
Human	0.04			Park *et al*. (2007)
Goat	0.1–0.4			Park *et al*. (2007)

SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay.

of different immunoglobulin classes in milk and colostrum varies considerably depending on species, breed, age, stage of lactation, and health status. The IgG content in yak milk shows large variations depending on species and season (Table 29.10). In mature yak milk the IgG content is higher than in human milk, which is very important for infants.

The growth factors epidermal growth factor (EGF), betacellulin (BTC), insulin-like growth factor (IGF)-I, IGF-II, TGF-β1, TGF-β2, fibroblast growth factor (FGF)-1, FGF-2, and platelet-derived growth factor (PDGF) are present in bovine milk and colostrum but are also found in human milk but at lower concentrations. The compositional data in the literature vary greatly and provide evidence that the day of lactation has the most important effect. Milk growth factors are characterized by a neutral to alkaline isoelectric point (pI) and a molecular mass between 6400 and 30 000 Da. However, many of the growth factors are in a latent form, bound to high-molecular-mass proteins. Lactoperoxidase is a member of the heme peroxidase family of enzymes, and together with its inorganic ion substrate, hydrogen peroxide, and oxidized products is known as the lactoperoxidase system. It plays an important role in the innate immune system by killing bacteria in milk and mucosal secretions (from linings of mostly endodermal origin, covered in epithelium, which are involved in absorption and secretion) and hence augmentation of the lactoperoxidase system may have therapeutic applications. TGF-β2 and lactoperoxidase are also present in yak milk, but IGF-I is not detected because of its low content (Table 29.11). In mature Maiwa yak milk, the content of TGF-β2 (76.41 ng/mL) is higher than in mature bovine milk (3–17 ng/mL) and in human milk (0.8–3.1 ng/mL) (Gauthier *et al*., 2006). The other growth factors in yak milk cannot be discussed because of a lack of sufficient information.

Among minor whey proteins, yak milk also contains proteose peptones like bovine and other milks. The proteose peptone fraction has been characterized as a mixture of heat-stable, acid-soluble (pH 4.6) phosphoglycoproteins insoluble in 12% trichloroacetic acid. The proteose peptone

Table 29.11. Minor protein contents of Maiwa Yak milk.

	Lactoferrin (μg/mL)	IgG (mg/mL)	IgA (ng/mL)	IgM (ng/mL)	Immunoglobulin (mg/mL)	TGF-β2 (ng/mL)	IGF-I (ng/mL)	Lactoperoxidase (U/mL)
N	36	35	31	24		34	34	41
Mean	3.53	0.201	12.57	56.87	0.201	76.41	ND	2.95
SD	2.74	0.012	2.98	40.03	0.012	43.66		1.24
Min.	0.50	0.058	1.08	7.12	0.058	22.68		6.43
Max.	11.92	0.358	76.56	151.55	0.358	261.18		1.04

N, number of Maiwai yak milk samples; TGF, transforming growth factor; IGF, insulin-like growth factor.
Source: based on data from Cheng (2011).

content of yak milk is about 13.71 mg/dL (He *et al*., 2012). The proteose peptone is divided into two fractions: non-hydrophobic and hydrophobic. The non-hydrophobic fraction is mainly composed of PP5 and has other components such as PP8 fast and PP8 slow, which derive from casein by proteolysis. The hydrophobic fraction is mainly composed of PP3, which is indigenous to milk. Analysis using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) has shown the molecular mass of PP3 from yak milk whey to be 28 kDa (He *et al*., 2012).

29.4.4 Milk fat globule membrane proteins

In all types of milk, fat globules are surrounded by a membrane named milk fat globule membrane (MFGM), which mainly comprises proteins and phospholipids. There are over 40 identified different proteins, ranging in molecular mass from 15 to 240 kDa, of which at least six are glycoproteins (McPherson *et al*., 1984). The major proteins have molecular masses of 155, 67, 50, and 49 kDa, identified as xanthine oxidase (XO), butyrophilin, periodic acid Schiff 6 (PAS 6), and periodic acid Schiff 7 (PAS 7), respectively (Mather, 2000).

Figure 29.2 presents the protein patterns of MFGM, isolated from Maiwa yak milk by SDS-PAGE (10% acrylamide) under reducing conditions (He *et al*., 2010). There are six major protein bands in yak milk MFGM, ranging in molecular mass from 47 to 224 kDa, the major band corresponding to mucin (MUC)-1 (225.6 kDa), the largest of the MFGM proteins. The other five MFGM proteins are XO (157.4 kDa), PAS III/IV (78–98 kDa), butyrophilin (67.5 kDa), PAS 6 (50.2 kDa) and PAS 7 (47.8 kDa) (He *et al*., 2010). For cow milk, the molecular mass of MFGM proteins ranges from 17 to 225 kDa depending on breed (Mather, 2000).

The protein content of yak milk MFGM is presented in Table 29.12. The content of MUC-1 in yak MFGM is 4.9%. MUC-1 plays an immunoprotective role by binding to and sequestering pathogenic microorganisms (*Escherichia coli*), preventing them from colonizing the intestinal tract (Peterson *et al*., 1998). Yak MFGM has a high content of XO, a major component of the MFGM proteins. XO can inhibit the growth of *Staphylococcus aureus*, *E. coli* and *Salmonella enteritidis*, based on hydrogen peroxide formation or stimulation of the

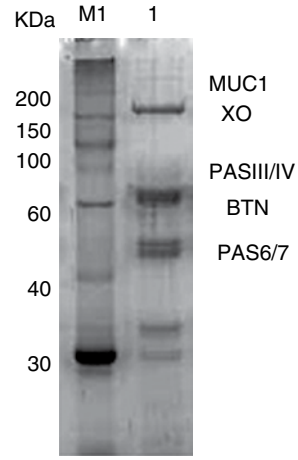


Figure 29.2. SDS-PAGE (10% acrylamide gel) of MFGM protein isolated from Maiwa yak milk. BTN, butyrophilin; MUC1, mucin 1; PAS, periodic acid Schiff; XO, xanthine oxidase.

Table 29.12. Protein content (%) of MFGM from Maiwa yak milk.

Milk fat globule membrane protein	Content
Mucin 1	4.87 ± 0.54
Xanthine oxidase	16.31 ± 0.10
Periodic acid Schiff III/IV	3.81 ± 0.45
Butyrophilin	25.43 ± 0.63
Periodic acid Schiff 6	13.45 ± 0.18
Periodic acid Schiff 7	13.46 ± 3.00
Other proteins	22.67 ± 3.00

Sources: based on data from Li *et al*. (2009) and He *et al*. (2010).

lactoperoxidase system in milk (Harrison, 2004; Martin *et al*., 2004). The content of PAS III/IV in yak MFGM is lower than that of other MFGM proteins. PAS III/IV is located in the outer layer of the MFGM and is easily displaced from the MFGM during abstraction and separation processes. Butyrophilin is the most abundant of the proteins comprising the MFGM in yak milk. Butyrophilin in milk could act as a molecular mimic of myelin oligodendrocyte glycoprotein (MOG), resulting in cross-reactivity (Guggenmos *et al*., 2004). Butyrophilin can modulate the encephalitogenic T-cell response to MOG in experimental autoimmune encephalomyelitis, which is related to human multiple sclerosis. Butyrophilin is a transmembrane protein of the outer layer, which is closely connected with XO. In MFGM, the action of butyrophilin is as an anchor point to form a supramolecular complex that interconnects the inner and outer membrane (Mather & Keenan, 1998). Together with adipophilin and XO, butyrophilin plays an important role in the assembly and stabilization of the MFGM (Mather, 2000). PAS 6/7 is the second most abundant of the proteins comprising yak MFGM. It is located in the outer part of the membrane and is heavily glycosylated.

29.4.5 Amino acids

The 22 standard amino acids are used to synthesize proteins and other biomolecules or are oxidized to urea and carbon dioxide as a source of energy. Of the 22 standard amino acids, eight are essential because the human body cannot synthesize them from other compounds at the level required for normal growth, so they must be obtained from food. In addition, histidine, cysteine, taurine, tyrosine, and arginine are semi-essential amino acids in children, because the metabolic pathways that synthesize these amino acids are not fully developed. The content of most individual amino acids and total amino acids in yak milk protein is higher compared with bovine, goat, and human milk due to the content of protein in yak milk, which is higher than in bovine, goat, and human milk (Table 29.13).

The Food and Agriculture Organization and the World Health Organization recommend that the ratio of total

Table 29.13. Amino acid content (g/100 g) of yak milk.

	Maiwa yak[1]	Gannan yak[1]	Bovine[2]	Goat[2]	Human[2]
Essential amino acid (EAA)					
Thr	0.18	0.21	0.15	0.16	0.05
Val	0.25	0.22	0.16	0.24	0.06
Met	0.11	0.13	0.06	0.08	0.02
Ile	0.23	0.2	0.14	0.21	0.06
Leu	0.42	0.46	0.29	0.31	0.10
Phe	0.21	0.23	0.16	0.16	0.05
Lys	0.37	0.39	0.27	0.29	0.07
His	0.11	0.11	0.10	0.09	0.02
Trp	0.06	0.06	0.05	0.04	0.02
Total EAA (TEAA)	1.94	2.00	1.33	1.58	0.45
Non-essential amino acid (NEAA)					
Cys	0.03	0.03	0.02	0.05	0.02
Arg	0.15	0.15	0.11	0.12	0.04
Pro	0.45	0.48	0.32	0.37	0.08
Asp	0.33	0.36	0.26	0.21	0.08
Ser	0.23	0.27	0.16	0.18	0.04
Glu	1.03	1.13	0.77	0.63	0.17
Gly	0.09	0.1	0.06	0.05	0.03
Ala	0.14	0.16	0.10	0.12	0.04
Tyr	0.20	0.22	0.15	0.18	0.05
Total NEAA (TNEAA)	2.66	2.92	1.95	1.91	0.55
Total amino acid (TAA)	4.60	4.91	3.33	3.49	1.00
TEAA/TNEAA	0.73	0.68	0.68	0.81	0.82
TEAA/TAA	0.42	0.41	0.40	0.45	0.45

Sources: [1]based on data from Li *et al*. (2011); [2]based on data from Guo & Luo (1992).

essential amino acids (TEAA) to total non-essential amino acids (TNEAA) and of TEAA to total amino acids (TAA) of protein should be above 60% and 40%, respectively. The ratio of TEAA/TNEAA and TEAA/TAA in yak milk protein is 73% and 42%. This result shows that the amino acid composition of yak milk protein is balanced and has high nutritional value.

The contents of amino acids is affected by season as shown in Table 29.14. From May to October, the content of individual amino acids and of total amino acids is not significantly different; however, from December the content of amino acids is lower compared with yak milk samples from May to October. This is consistent with changes in total protein content from different months (Table 29.2). From May to December, the ratios of TEAA/TNEAA and TEAA/TAA gradually decrease. The ratio of TEAA/TNEAA is related to the protein ratio of herbage grazed by the yaks (Foldager *et al*., 1980). Because yaks are allowed to graze naturally at an average elevation of 3600 m on the Qinghai-Tibet Plateau, the important factors affecting milk quality are pasture production and the quantity, growth status and nutritive value of the herbage. All lactating yaks, irrespective of age, parity or breed type, or even location, tend to peak in yield in the summer season (June to August), when grass is at its best quality and quantity, while after August, as air temperatures fall, the nutritive value declines (Wiener *et al*., 2003).

29.4.6 Bioactive peptides derived from yak milk proteins

Enzymatic hydrolysis of milk proteins can release fragments able to exert specific biological effects, such as antihypertensive, antimicrobial, opioid, antioxidant, immunomodulant, or mineral-binding activities. Such protein fragments, known as bioactive peptides, are formed from the precursor inactive protein during gastrointestinal digestion and/or

Table 29.14. Amino acid content (g/100 g) of yak milk from different seasons.

	Warm season		Cold season	
	May	**September**	**October**	**December**
Essential amino acid (EAA)				
Thr	0.22 ± 0.01^{c}	0.19 ± 0.01^{b}	0.19 ± 0.01^{b}	0.15 ± 0.02^{a}
Val	0.31 ± 0.02^{c}	0.25 ± 0.01^{b}	0.25 ± 0.02^{b}	0.20 ± 0.03^{a}
Met	0.12 ± 0.01^{b}	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.09 ± 0.02^{a}
Ile	0.28 ± 0.02^{c}	0.23 ± 0.01^{b}	0.24 ± 0.02^{b}	0.18 ± 0.03^{a}
Leu	0.47 ± 0.03^{b}	0.42 ± 0.01^{b}	0.43 ± 0.02^{b}	0.33 ± 0.06^{a}
Phe	0.23 ± 0.01^{b}	0.21 ± 0.01^{b}	0.22 ± 0.02^{b}	0.17 ± 0.03^{a}
Lys	0.44 ± 0.03^{c}	0.37 ± 0.01^{b}	0.38 ± 0.03^{b}	0.29 ± 0.05^{a}
His	0.13 ± 0.01^{c}	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.09 ± 0.01^{a}
Total EAA	2.20	1.89	1.95	1.50
Non-essential amino acid (NEAA)				
Cys	0.05 ± 0.01^{c}	0.03 ± 0.01^{a}	0.04 ± 0.01^{b}	0.03 ± 0.01^{a}
Arg	0.17 ± 0.01^{c}	0.15 ± 0.01^{b}	0.16 ± 0.01^{b}	0.12 ± 0.02^{a}
Pro	0.44 ± 0.02^{b}	0.45 ± 0.01^{b}	0.51 ± 0.04^{c}	0.37 ± 0.05^{a}
Asp	0.38 ± 0.03^{c}	0.33 ± 0.01^{b}	0.32 ± 0.02^{b}	0.26 ± 0.04^{a}
Ser	0.24 ± 0.02^{b}	0.23 ± 0.01^{b}	0.23 ± 0.02^{b}	0.19 ± 0.03^{a}
Glu	1.15 ± 0.06^{c}	1.03 ± 0.02^{b}	1.06 ± 0.08^{b}	0.83 ± 0.12^{a}
Gly	0.00 ± 0.00^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.35 ± 0.05^{b}
Ala	0.12 ± 0.01^{a}	0.14 ± 0.01^{b}	0.16 ± 0.01^{b}	0.11 ± 0.02^{a}
Tyr	0.23 ± 0.01^{b}	0.20 ± 0.01^{b}	0.22 ± 0.02^{b}	0.16 ± 0.04^{a}
Total NEAA (TNEAA)	2.78	2.65	2.79	2.42
Total amino acid (TAA)	4.98	4.54	4.74	3.92
TEAA/TNEAA	0.79	0.71	0.70	0.62
TEAA/TAA	0.44	0.42	0.41	0.38

Within a row, means lacking common lower case superscripts differ ($P < 0.05$).

Source: courtesy of H. Li. Reproduced from Li (2011), with permission.

during food processing (Korhonen & Pihlanto-Leppälä, 2003). Because of their physiological and physicochemical versatility, milk peptides are regarded as highly prominent components for health-promoting foods or pharmaceutical applications.

Among the known bioactive peptides, those with angiotensin-converting enzyme (ACE)-inhibitory properties have received special attention due to their potentially beneficial effects in the treatment of hypertension. ACE is a multifunctional enzyme, located in different tissues, and able to regulate several systems that affect blood pressure. It is responsible for generating vasopressor angiotensin II and inactivation of the vasodepressor bradykinin.

Caseins are also an important source of peptides with ACE-inhibitory activity after enzymatic hydrolysis. Mao *et al*. (2007) used alcalase to hydrolyze yak milk casein and obtained two novel ACE-inhibiting peptides PPEIN (κ-CN f156–160) from κ-CN and PLPLL (β-CN f136–140) from β-CN. The ACE-inhibitory activity of yak milk casein hydrolyzate varies with hydrolysis time. The molecular masses of the purified ACE inhibitors PPEIN and PLPLL from yak casein hydrolyzate are 550 and 566.4 Da, and their amino acid sequences are Pro-Pro-Glu-Ile-Asn (PPEIN) and Pro-Leu-Pro-Leu-Leu (PLPLL), respectively. The IC_{50} values of PPEIN and PLPLL are 0.29 ± 0.01 and 0.25 ± 0.01 mg/mL, respectively.

Enzymatic hydrolysis of yak casein by various proteases results in the release of bioactive peptides with potential free radical-scavenging and anti-inflammatory properties. Mao *et al*. (2011) evaluated the antioxidative and anti-inflammatory activities of yak milk protein and their alcalase and trypsin hydrolyzates. The results indicate that yak casein hydrolyzate can reduce free radical production, and the 7-hour hydrolyzate prepared with alcalase shows the highest anti-radical activity. It can substantially decrease the production of nitric oxide and the proinflammatory cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in lipopolysaccharide-stimulated murine peritoneal macrophages, which shows that it has anti-inflammatory activity.

29.5 LIPIDS

Lipids are the most important components of milk in terms of cost, nutrition, and the physical and sensory characteristics they impart to dairy products. Fat is dispersed in milk in the form of spherical droplets called milk fat globules. The size distribution of fat globules varies between milk species (Mehaia, 1995). Like other animal lipids, the lipid composition of yak milk consists of simple lipids (diacylglycerols, monoacylglycerols, and cholesterol esters), complex lipids (phospholipids), and liposoluble compounds (sterols, cholesterol esters, hydrocarbons) (He, 2012).

The fatty acid profile has received more attention due to its relation to fat nutrition. The unsaturated fatty acids (USFAs) are perceived to be healthier than saturated fatty acids (SFAs). Monounsaturated fatty acids (MUFAs) and certain polyunsaturated fatty acids (PUFAs) play an important role in the prevention of cardiovascular diseases, hypertension, diabetes, arthritis and other inflammatory or autoimmune disorders, and cancer (Ulbricht & Southgate, 1991; Simpolus, 1999). Conjugated linoleic acids (CLA), a naturally formed group of positional and geometric isomers of linoleic acid, have attracted scientific interest due to their potential beneficial effects in the prevention and treatment of atherosclerosis, carcinogenesis, and obesity (Wahle & Heys, 2002). *Cis*-9, *trans*-11 is the major isomer of CLA in milk fat and accounts for 80–90% of the total CLA. It can be produced by the incomplete biohydrogenation of linoleic acid in the rumen, but is mainly synthesized from *trans*-11 C18:1 by desaturation in the mammary gland (Werner *et al*., 1992; Griinari *et al*., 2000).

The composition of fatty acids is influenced by intrinsic factors, such as breed, stage of lactation, and animal health, and also by extrinsic factors, such as feeding system, seasonal changes, environmental conditions, and management (Lindmark-Mansson *et al*., 2003). The content and profiles of fatty acids in yak, cow, goat, and sheep milk are listed in Table 29.15. Like all types of milk, the predominant fatty acids in yak milk are palmitic (C16:0), followed by oleic (C18:1), stearic (C18:0), and myristic (C14:0). SFAs constitute 63.4–64.5% of total fatty acids in yak milk. In the SFA class, a lower proportion of myristic acid (C14:0) in yak milk seems to be favorable for human health because of its negative role in atherosclerosis (Pfeuffer & Schrezenmeir, 2000). Yak milk contains high proportions of MUFAs and PUFAs, indicating that yak milk has high nutritional properties, because MUFAs and PUFAs play important roles in the prevention and treatment of cardiovascular diseases, hypertension, diabetes, arthritis and other inflammatory or autoimmune disorders, and cancer (Ulbricht & Southgate, 1991). In the PUFA category, the content of *cis*-9, *trans*-11 CLA in yak milk is almost twice that in cow, goat and sheep milk. The high content of CLA in yak milk indicates that yak milk has very good nutritional value for human health, because CLA has been shown to have different physiological effects, such as reducing body fat accretion, preventing the development of atherosclerosis, enhancing bone mineralization, and exhibiting antidiabetic, anticarcinogenic, cholesterol-depressing, antioxidative, growth-promoting and immune-modulating activities (Pariza *et al*., 2001). Yak milk contains high amounts of α-linolenic acid (C18:3), which is beneficial to human health by preventing cardiovascular diseases and hypertension (Parodi, 2004). Compared with

Table 29.15. Fatty acid composition (g/100 g of total fatty acids) of yak and other mammalian milks.

Fatty acid	Maiwa yak[1]	Zhongdian yak[2]	Gannan yak[2]	Bovine[3]	Goat[3]	Sheep[3]
C6:0	1.11 ± 0.25	0.96 ± 0.16	0.93 ± 0.10	2.21	2.40	2.78
C8:0	0.70 ± 0.12	0.69 ± 0.13	0.56 ± 0.02	2.32	2.53	3.13
C10:0	1.50 ± 0.23	1.60 ± 0.18	1.19 ± 0.36	3.52	9.38	4.97
C12:0	1.31 ± 0.31	1.74 ± 0.17	1.16 ± 0.11	1.97	4.45	3.35
C13:0	0.09 ± 0.03	0.05 ± 0.01	0.08 ± 0.01	—	—	—
iso-C14:0	0.24 ± 0.05	0.21 ± 0.03	0.24 ± 0.05	—	—	—
C14:0	7.41 ± 0.82	7.86 ± 1.18	7.54 ± 1.10	11.41	10.16	10.16
C14:1	0.67 ± 0.11	0.77 ± 0.12	0.71 ± 0.12	0.84	0.22	0.58
iso-C15:0	0.73 ± 0.27	0.42 ± 0.02	0.48 ± 0.13	—	—	—
C15:0	1.56 ± 0.25	1.02 ± 0.16b	1.55 ± 0.23	—	—	—
iso-C16:0	0.54 ± 0.10	0.43 ± 0.12	0.52 ± 0.14	—	—	—
anteiso-C16:0	0.53 ± 0.11	0.23 ± 0.02	0.27 ± 0.09	—	—	—
C16:0	27.13 ± 2.57	25.36 ± 2.55	25.08 ± 2.31	25.59	24.20	23.11
cis-9 C16:1	1.99 ± 0.25	1.37 ± 0.25	1.62 ± 0.19	1.68	0.67	0.39
trans-9 C16:1	0.26 ± 0.08	0.23 ± 0.02	0.26 ± 0.09	0.31	0.38	0.29
iso-C17:0	0.76 ± 0.15	0.62 ± 0.13	0.85 ± 0.11	—	—	—
C17:0	1.33 ± 0.31	0.96 ± 0.19	1.41 ± 0.35	0.54	0.63	0.76
C18:0	17.78 ± 2.14	20.62 ± 1.22	20.37 ± 2.31	11.82	12.51	12.86
trans-9 C18:1	0.34 ± 0.10	0.39 ± 0.11	0.58 ± 0.11	—	—	—
trans-11 C18:1	5.18 ± 0.47	6.60 ± 0.10	5.17 ± 0.82	2.01	1.69	2.69
cis-11 C18:1	0.17 ± 0.04	0.12 ± 0.01	0.13 ± 0.01	—	—	—
cis-9 C18:1	21.76 ± 1.07	19.53 ± 1.66	22.81 ± 2.10	24.72	22.03	23.72
cis-12 C18:1	0.64 ± 0.12	0.50 ± 0.09	0.51 ± 0.07	—	—	—
cis-9, *cis*-12 C18:2	1.80 ± 0.52	1.24 ± 0.15	1.15 ± 0.33	1.96	0.70	1.17
cis-9, *trans*-11 C18:2 (CLA)	1.02 ± 0.24	1.07 ± 0.12	0.89 ± 0.33	0.59	0.43	0.60
C18:3 *n*-3	1.39 ± 0.19	2.21 ± 0.17	1.32 ± 0.26	0.70	0.82	0.92
C19:0	0.21 ± 0.09	0.11 ± 0.04	0.32 ± 0.06	—	—	—
C19:1	0.32 ± 0.07	0.26 ± 0.07	0.21 ± 0.04	—	—	—
C20:0	0.70 ± 0.27	0.61 ± 0.11	0.57 ± 0.11	—	—	—
C20:1	0.21 ± 0.07	0.26 ± 0.05	0.22 ± 0.03	—	—	—
C20:4	0.16 ± 0.05	0.15 ± 0.01	0.12 ± 0.03	0.21	0.32	0.20
C20:5 *n*-3 (EPA)	0.07 ± 0.03	0.08 ± 0.06	0.05 ± 0.01	0.15	0.11	0.09
C21:0	0.10 ± 0.03	0.14 ± 0.02	0.22 ± 0.04	—	—	—
C22:0	0.22 ± 0.07	0.16 ± 0.01	0.21 ± 0.04	—	—	—
C22:6 *n*-3 (DHA)	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.08	0.09	0.08
SFAs	63.95 ± 6.67	64.49 ± 3.43	63.35 ± 2.69	63.94	68.70	65.17
MUFAs	31.54 ± 3.09	30.69 ± 1.03	32.97 ± 1.35	27.23	23.29	24.29
PUFAs	4.51 ± 0.65	4.82 ± 0.62	3.68 ± 0.70	3.24	2.52	3.12
SCFAs	3.31 ± 0.24	3.25 ± 0.33	2.68 ± 0.31	8.05	14.31	10.88
MCFAs	42.46 ± 4.75	39.69 ± 3.85	39.51 ± 4.13	42.69	39.70	42.56
LCFAs	54.23 ± 5.37	57.06 ± 6.07	57.81 ± 5.91	49.26	45.99	46.56
USFAs/SFAs	0.56 ± 0.12	0.55 ± 0.11	0.58 ± 0.07	0.48	0.38	0.42
AI	1.65 ± 0.18	1.69 ± 0.14	1.72 ± 0.09	2.20	2.57	2.21

SFAs, sum of all saturated fatty acids; MUFAs, sum of all monounsaturated fatty acids; PUFAs, sum of all polyunsaturated fatty acids; SCFAs, total short-chain fatty acids (C6–C11); MCFAs, total medium-chain fatty acids (C12–C16); LCFAs, total long-chain fatty acids (C16–C22); USFAs/SFAs, total unsaturated fatty acids/total saturated fatty acids.

$$AI = [C12:0 + (4 \times C14:0) + C16:0] / (C14:1 + C16:1 + C17:1 + C18:1 + C18:2 + C18:3)$$

Sources: [1] based on data from He (2012); [2] reproduced from He *et al*. (2011), with permission of Elsevier; [3] reproduced from Talpur *et al*. (2006), with permission of Elsevier.

cow, goat and sheep milk, yak milk has a low content of short-chain fatty acids (C6 to C12) and a high content of long-chain fatty acids (Noble, 1978).

The ratio of total USFAs to total SFAs can be used to evaluate the nutritional property of milk. A high USFA/SFA ratio can increase plasma very low density lipoprotein lipids and reduce the hepatic hypertriglyceridemic effect of dietary cholesterol (Chang *et al*., 2004). Some SFAs in milk have the potential to induce coronary heart disease, which can be evaluated by the index of atherogenicity (IA), calculated with the equation:

$$IA = [C12{:}0 + (4 \times C14{:}0) + C16{:}0] / (C14{:}1 + C16{:}1 + C17{:}1 + C18{:}1 + C18{:}2 + C18{:}3)$$

The value of IA shows a positive correlation with the onset of coronary heart disease, which is principally due to the obstruction of coronary vessels by atherosclerosis (Wahle & Heys, 2002). IA is proposed to better take into account the effects of different foods and diets on human health. A high IA value reflects the risk of cardiovascular disease resulting from lipid intake. The IA can indicate the health condition of different foods and diets, so the low IA value in yak milk shows that it is healthy for humans.

29.6 MINERALS

Yak milk and cow milk have similar ash content, around 0.8%, but the major mineral contents of yak milk are much higher than those in cow milk, while the content of phosphorus is in the range of cow milk (Table 29.16). Yak milk contains about 1500 mg/kg of Ca and 950 mg/kg of P, while human milk has only one-fifth to one-third as much of these two major minerals (Park *et al*., 2007). Dietary Fe is required for a wide variety of biochemical processes. Human milk, as well as bovine milk and milk products, are poor sources of Fe. To prevent Fe deficiency and anemia in infants aged 6–9 months, most infant formulae are supplemented with Fe (McSweeney & Fox, 2009). The higher Fe content of yak milk (0.57–1.25 mg/kg) could be of nutritional benefit in infant foods. The content of minerals varies depending on breed, diet, individual animal, stage of lactation, and status of udder health. The contents of Cu and Fe in Gannan yak milk are higher than those in Maiwa yak milk, but the content of Zn in Maiwa yak milk is higher than that in Gannan yak milk. In general, the mineral content of yak milk seems to vary much more than that of cow milk due to the monthly differences in feeding. The minerals in yak milk have not been extensively studied, even though they may be of considerable interest with regard to human nutrition and health.

29.7 VITAMINS

Yak milk has higher amounts of vitamin D and vitamin B_6 than bovine milk. The higher content of vitamin D is related to the grazing environment: because yak are found on the altiplano, they are exposed to ultraviolet irradiation for long periods. The levels of folate and vitamin B_{12} in yak milk are very low and cannot be detected by the HPLC method. The levels of vitamins in milk are highly dependent on the amount consumed in the feed. Vitamin concentrations of milk follow a seasonal trend, with higher values obtained during the outdoor grazing period (McSweeney & Fox, 2009). For bovine milk, higher concentrations of vitamins are present in fresh retail milk during both outdoor grazing (June to October) and indoor feeding (December to March) compared with manufactured milk, which reflects the higher food concentrate input into retail milk production (McSweeney & Fox, 2009). Because yak are natural grazers on the Qinghai-Tibetan Plateau, the vitamin contents have larger variations between breeds and seasons (Tables 29.17 and 29.18); however no summary of vitamin profiles of yak milk is possible due to limited data.

Table 29.16. Mineral content (mg/kg) of yak and other mammalian milks.

	Maiwa yak[1]	Gannan yak[1]	Bovine[2]	Goat[3]	Sheep[3]	Human[3]
Cu	0.45 ± 0.08	0.65 ± 0.02	0.1–0.6	0.5	0.4	0.6
Mg	154.10 ± 13.22	150.59 ± 13.98	90.00–140.00	160	180	40
Zn	7.31 ± 0.44	1.76 ± 0.33	2.00–6.00	5.6	5.7	3.8
Fe	0.57 ± 0.04	1.25 ± 0.05	0.012–0.035	0.7	0.8	2.0
Mn	0.06 ± 0.01	0.02 ± 0.02	0.16–0.35	0.32	0.07	0.7
Ca	1545.45 ± 145.61	1525.2 ± 177.0	1000.00–1300.00	1340	1930	330
P	922.04 ± 70.13	1023.9 ± 81.2	900.00–1000.0	1210	1580	430

Sources: [1]based on data from Li *et al*. (2011); [2]reproduced from Heck *et al*. (2009), with permission of Elsevier; [3]reproduced from Park *et al*. (2007), with permission of Elsevier.

Table 29.17. Vitamin content (μg/100 g) of yak and other mammalian milks.

	Maiwa yak[1]	Gannan yak[1]	Bovine[2]	Goat[3]	Sheep[3]	Human[3]
Vitamin B_1	48.54 ± 11.54	23.56 ± 11.29	45.00	68.00	80.00	17.00
Vitamin B_2	79.49 ± 28.15	20.79 ± 5.74	175.00	210.00	376.00	20.00
Vitamin B_3	2.61 ± 3.21	1.58 ± 0.67	90.00	0.31	0.41	0.20
Vitamin B_6	40.75 ± 16.21	0.36 ± 0.18	0.23	0.05	0.08	0.01
Vitamin A	13.88 ± 4.52	89.79 ± 4.72	47.74	59.20	46.72	60.80
Vitamin D	0.15 ± 0.21	3.95 ± 0.30	0.06	0.06	0.18	0.04
Vitamin E	30.15 ± 7.30	91.85 ± 21.25	100.00	—	—	—

Sources: [1]based on data from Li *et al*. (2011); [2]based on data from Guo (2011); [3]reproduced from Park *et al*. (2007), with permission of Elsevier.

Table 29.18. Vitamin content (μg/100 g) of Maiwa yak milk from different seasons.

	May	September	October	December
Vitamin B_1	47.09 ± 4.03	37.04 ± 5.64	50.05 ± 11.21	63.65 ± 12.08
Vitamin B_2	1.06 ± 0.56	148.56 ± 9.33	1.27 ± 0.64	117.89 ± 20.68
Vitamin B_3	0.11 ± 0.03	0.51 ± 0.72	3.71 ± 3.77	6.05 ± 0.82
Vitamin B_6	0.90 ± 0.31	0.34 ± 0.18	0.45 ± 0.11	0.13 ± 0.04
Vitamin A	1.12 ± 0.32	25.04 ± 0.96	4.07 ± 4.49	17.28 ± 0.74
Vitamin D	0.40 ± 1.22	0.25 ± 0.09	0	—
Vitamin E	1.59 ± 1.74	36.69 ± 17.20	2.53 ± 2.41	68.08 ± 3.21

Source: based on data from Li *et al*. (2011).

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30
Other Minor Species Milk (Reindeer, Caribou, Musk Ox, Llama, Alpaca, Moose, Elk, and Others)

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30.1 INTRODUCTION

Generally, there is a paucity of scientific information on the milk of minor species such as reindeer, caribou, musk ox, llama, alpaca, and others besides minor domesticated species such as sheep, goats, buffaloes, camel, yak, mithun, horse, and donkey. Since the milk of some of these mammals is produced for human consumption only in certain parts of the world and only limited studies on their milk have been conducted, the availability of research data has been scarce. However, their milk is important for the nutritional and economic well-being of people in certain regions of the world, where production of cow milk is limited or impossible for climatic reasons.

The composition of the milk of wild and domesticated minor species may vary widely due to lack of sample numbers, difficulties in defining the stage of lactation, bias introduced during sampling, and different analytical procedures (Oftedal, 1984; Park, 2011). Milk products from these species are small in numbers, but they are unique to the needs of people in special regions of the world, where they significantly contribute to the nutritional and economic well-being of these people. This chapter discusses the milk of reindeer, caribou, musk ox, llama, alpaca, moose, elk, and other minor species in comparison to human milk.

On the other hand, a distinction is necessary: some domesticated minor species, such as goat, sheep, camel, buffalo, mithun, yak, horse, donkey, reindeer, and moose, are managed also for milk production, but except for a few goat and sheep breeds are not "dairy" species; furthermore, there are no wild dairy species, nor are the "other minor" species discussed in this book, such as sows, caribou, musk ox, elk, pinnipeds, polar bear, and elephant, managed for milk production except under experimental research conditions.

30.2 GENERAL ASPECTS OF MILK OF MINOR SPECIES

The specific chemical composition of milk of different domesticated or wild species is designed by natural selection to provide the nutritional needs of the neonate of the specific species (Park, 2006). There are considerable differences in the basic composition of milk among different domesticated and wild mammals (Table 30.1).

Differences in milk composition between minor species can be erroneous or misleading due to the unknown stage of lactation and time of sampling from the gland, resulting in confounding effects on the composition of the milk (Oftedal, 1984). Even under standard conditions of milk sampling, there are substantial short-term (diurnal and day-to-day)

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Table 30.1. Average composition (g/dL) of milk of minor mammalian species.

Mammals	Days of lactation	No. samples	Total solids	Protein	Lactose	Fat	Ash
Ass	60–120	9	9.1	1.6	6.2	1.0	0.4
Bison	?	2	13.7	4.2	4.7	1.7	1.0
Caribou	?	3	23.6	7.6	3.7	11.0	1.3
Dromedary	?	15	13.6	3.6	5.0	4.5	0.7
Elk	14–77	28	19.0	5.7	4.2	6.7	1.3
Llama	2–120	54	13.1	3.4	6.5	2.7	0.5
Moose	>2	15	23.6	11.0	3.3	8.5	1.5
Musk ox	—	1	19.0	5.3	3.8	8.2	1.7
Reindeer	4–5	8	27.1	11.1	3.0	11.1	1.5
Fur seal*	20–180	83	57.4	12.1	—	42.8	—

*Sub-antarctic fur seals; based on data from Georges *et al*. (2011).
Source: based on data from Park (2006).

variations in composition, due to environmental conditions, feeding, management, season, locality, disease, and yield per day, as is also the case for the major domestic milk producers the cow, buffalo, goat, sheep, camel, and yak (Schmidt, 1971; Park, 2006).

As in the case of cow and other major dairy species, the colostrum of all minor domesticated and wild mammalian species contains much higher levels of total solids, protein, and minerals than the mature milk obtained 2 or 3 weeks after parturition. The high protein content in colostrum is due to globulins, which contain antibodies. Since the antibody titer of blood of the newborn is low, mammals such as cow, sheep, goat, horse, and pig require passive immunity from colostrum and its immunoglobulins (Schmidt, 1971; Park, 2011).

The milk of most wild mammals contains much higher levels of major nutrients including protein, fat and minerals than the milk of major dairy species such as cow and goat. Dietary roughage is important to maintain the level of milk fat in ruminant milk. A decrease in roughage intake depresses the milk fat content, causes changes in rumen fermentation, and possibly even parakeratosis. Research has shown that at least 17% crude fiber is needed in ruminant diets to prevent a decrease in molar percentage of acetic acid and increase in propionic acid in the rumen, which causes a low fat content in milk (Schmidt, 1971).

30.3 PRODUCTION, COMPOSITION, AND UTILIZATION OF MILK FROM MINOR DAIRY SPECIES

30.3.1 Reindeer

At least 2000 years ago, reindeer husbandry and milking evolved in the taiga region of eastern Siberia around Lake Baikal and spread to nearby ethnic groups (Fondahl, 1989). Cultural exchange and expansion of pastoral nomads living on the northern fringe of the Asian steppe developed reindeer milking along the borders of Russia, Mongolia, and China (Fondahl, 1989). The famous horse and cattle breeders, the Yakuts, adapted reindeer raising as they migrated north and introduced an advanced milking culture into the region (Fondahl, 1989; Holand *et al*., 2006). In addition, in Scandinavia the Nordic Saami people of Lapland evolved reindeer milking independently. In the late 1800s, Saami reindeer herding families practiced small-scale reindeer pastoralism and the milk was manufactured into cheese and butter for their own consumption and for sale (Holand *et al*., 2006).

Reindeer/caribou (*Rangifer tarandus*) has a "follower" type mother–young relationship characterized by great seasonal mobility (Geist, 1999). Calving occurs at the end of the northern winter and the lactation period usually terminates during the rutting/breeding season in October (Holand *et al*., 2002). Reindeer (*Rangifer tarandus tarandus*) and caribou (*Rangifer tarandus granti*) are biologically closely related and have ancient associations in northern lands. Reindeer/caribou have evolved in a harsh environment with a short summer season, suggesting rapid and efficient transmission of energy and protein from the mother to the calf to optimize lifetime reproductive success (Jönsson, 1997).

Although it depends on latitude and environmental conditions, reindeer milking usually begins a month after calving during May, and continues up to the rut in late September and early October (Holand *et al*., 2006). Among advanced south-eastern Siberian reindeer herders, the calves and females are normally tethered during alternate periods to ensure that both remain close to the campsite, but are kept separated during part of the day. The females are milked up to three times daily and the period of separation of the calf from the mother varies with stage of lactation (Holand *et al*., 2006; Park, 2011).

30.3.1.1 Production of reindeer milk

The lactation curve of reindeer is similar to that of other ungulates (Holand *et al*., 2002). The milk yield of reindeer is significantly affected by week of lactation and individual animals, and the lactation curve has an asymmetrical peak at 3 weeks postpartum. Milk yield at peak lactation was reported as 983 g/day (range 595–1239 g/day) (Fig. 30.1; Gjostein *et al*., 2004). The length of lactation varied from 24 to 26 weeks and average total milk production was 99.5 kg. From peak lactation, milk production decreased linearly until it terminated. The energy output averaged 7996 kJ/day at peak lactation, and significantly decreased to the end of lactation.

The potential milk production of reindeer and caribou has been studied using isotope tracer techniques, which are accurate but elaborate and expensive (Holand *et al*., 2006; Park, 2011). The estimated total milk yield of reindeer is lower than that of red deer (150 kg) and Iberian red deer (224 kg), but slightly higher than that of black tailed deer (93 kg) (Holand *et al*., 2006).

30.3.1.2 Nutritional composition of reindeer milk

Although it is difficult to present a typical composition of reindeer milk due to variations in sampling, feeding regime, and lactation stage among different studies, wild and semi-domestic ruminants generally produce richer milk, particularly in late lactation, than domesticated species (Park, 2006, 2011). Reindeer milk at peak and mid lactation has a relatively high fat (11–15%) and protein (7–10%) content, but a moderately low level of lactose (about 3.5%) (Holand *et al*., 2006; Table 30.2). Reindeer milk has a high milk solids content compared with other ungulates and undergoes great compositional changes during a relatively short lactation cycle (Luhtala *et al*., 1968; Robbins *et al*., 1987).

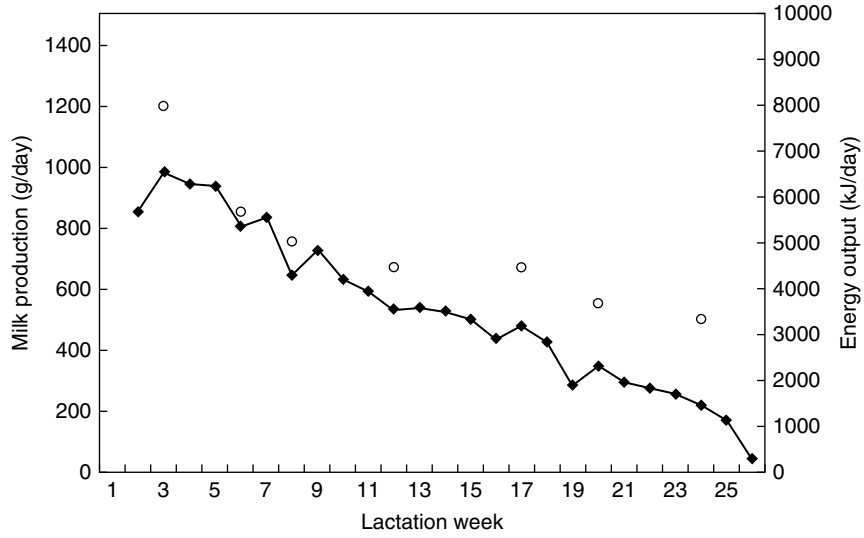


Figure 30.1. Mean milk production (solid symbols, g/day) and total energy output (open symbols, kJ/day) during lactation in reindeer (*Rangifer tarandus*). The milk production data are based on means of 2 years' study. Reproduced from Gjostein *et al.* (2004), with permission of Elsevier.

Table 30.2. Gross composition (%) of reindeer milk from peak lactation for 3–5 weeks postpartum.

Week of lactation	No.	Total solids	Fat	Protein	Lactose	Ash
4–5	8	27.1	11.1	11.1	3.0	1.5
4	3	23.7	10.2	7.5	3.7	1.2
5	5	38.1	19.6	13.0	3.7	2.7
3–5	7	31.6	15.5	10.7	3.7	1.3
5	2	32.8	17.1	10.9	2.8	1.5

Source: based on data from Holand *et al*. (2006).

Mean content of fat, protein, and lactose of reindeer milk were reported as 15.5, 9.9, and 2.5%, respectively, by Gjostein *et al*. (2004). The fat and protein contents increased markedly with stage of lactation and there was a decrease in the protein/fat ratio as protein was substituted by fat with advancing stage of lactation. The caloric value of the milk averaged 8.7 kJ/g and increased significantly with stage of lactation.

Average protein content of reindeer milk is around 9% in early lactation and about 11% in late lactation, while the relative percentages of different amino acids are rather constant throughout lactation (Luhtala *et al*., 1968). The amino acid profile resembles that in sheep and goat milk, except for low cysteine and high tyrosine content. Casein is the predominant protein fraction in reindeer milk (7–9%). β-Lactoglobulin is the main whey protein, comprising162 amino acids (Rytkönen *et al*., 2002). Reindeer milk contains abundant non-protein nitrogen (NPN), urea (about 48 mg/dL), ammonium, carnitine (84–118 mg/dL) and free carnitine (about 71 mg/L) (Malinen *et al*., 2002).

The major energy source of reindeer milk is fat, which represents 67% of the energy content at peak lactation and 75% at late lactation (Gjostein *et al*., 2004). Fatty acids of reindeer milk are dominated by palmitic acid (16:0), which accounts for one-third of the total fatty acids, and stearic (18:0), oleic (18:1), and myristic (14:0) acids, which each contribute around 13%. The levels of short-chain fatty acids, especially butyric (4:0) and capric (6:0), are higher in reindeer than in red deer, roe deer, and fallow deer milk (Csapó *et al*., 1987). Reindeer milk contains about 3–3.5% lactose at peak lactation and this is lower than in other wild ungulates; it also contains small amounts of oligosaccharides.

Reindeer milk contains moderate to high levels (1–1.5%) of minerals compared with most other ungulates (Luhtala *et al*., 1968; Csapó *et al*., 1987). Reindeer milk is high in fat- and water-soluble vitamins. The vitamin C content is around 2 mg/dL, which is similar to that in the milk of red deer and fallow deer. Reindeer milk contains several times higher amounts of vitamin D_3 (0.5–2.0 mg/kg) than bovine milk (Luhtala *et al*., 1968; Csapó *et al*., 1987), and about twice as high levels of potassium (0.06–0.08 mg/kg) (Csapó *et al*., 1987).

30.3.1.3 Contribution of reindeer milk to human foods

Reindeer milk is produced and consumed in fluid and processed products. Children of reindeer herders drink fresh milk while adult people consume it in tea and coffee. The milk is also powdered and/or processed into cheese, butter, and sour cream, and is also used in medications (Table 30.3). The milk is curdled and often mixed with tasty herbs (*Oxyria* spp. and *Angelica* spp.). Reindeer milk is also stored frozen and often mixed with berries (*Vaccinium* spp., *Empetrum nigrum*) (Aikio *et al*., 2002).

Normally, reindeer milk from the first stage of lactation is consumed fresh, that from the second stage of lactation is used mainly for cheese production, while milk from the last stage of lactation is used more appropriately for churning butter. Reindeer milk and its products are highly priced, and are also used for medical purposes such as cures for digestive problems due to its antidiarrheal properties and for healing wounds. Fat oozed by heat from reindeer cheese is used to cure nursing pains, frostbite, and other injuries. Colostrum is used for children's ailments (Fondahl, 1989; Holand *et al*., 2006).

30.3.2 Caribou

As cervids, caribou (*Rangifer tarandus granti*) and reindeer (*Rangifer tarandus tarandus*) are biologically very similar and share ancient association in northern lands. Caribou

Table 30.3. Traditional products and uses of reindeer milk.

Fresh milk
Consumed by children often diluted with water, used in tea and coffee and in medical treatments
Stored milk
Frozen: stored for consumption during winter, ice cream mixed with berries
Fermented
Short: sour cream and cultured milk inoculated by a bacterial starter, for consumption
Long: stored in wooden containers often mixed with herbs, curdled, consumed during winter and spring migration, both the liquid and solid phase
Dried: tent dried in stomach compartment (reticulum) for a longer period, winter consumption
Manufactured milk
Cheese: curdled by heating or dried abomasums, dried and stored for consumption and sale
Butter: churned both from fresh and fermented milk, consumption and sale
Other products: buttermilk and whey, consumed fresh and reduced and eaten as soup

Source: reproduced from Holand *et al*. (2006), with permission of Blackwell Publishing.

have provided the indigenous northern Americans with meat, skins for clothing and shelter of exceptional warmth and lightness, and implements made from bones and antlers (Irving, 1975), and were also exploited for the fur trade.

Several different groups of caribou exist in North America, where some herds have different migratory patterns (Jernsletten & Klokov, 2002). The Porcupine herds have major migration routes between Canada and Alaska at least twice annually. The Nelchina herd ranges over south central Alaska, which is a diversified area of ragged glacier-capped mountains, rolling uplands, and broad forested plains. The Kaminuriak population ranges over barren grounds of northern Manitoba, north-eastern Saskatchewan, south-eastern District of Keewatin, and the Northwest Territories of Canada (Hemming, 1975; Jernsletten & Klokov, 2002).

Nursing is usually terminated during the rutting/breeding period for well-conditioned caribous. The neonates of caribou and musk oxen are followers, accompanying their mothers most of the time, and nursing is characterized by frequent nursing bouts of short duration (Parker *et al*., 1990).

Caribou milk has significantly higher protein, dry matter, and energy content than that of musk oxen (Table 30.1 and Fig. 30.2). Parker *et al*. (1990) attributed these compositional differences to the time of weaning at the end of summer. The caribous wean their calves in early October, whereas musk oxen continue to nurse their young until the third week of January. Milk intake by caribou neonates gradually declines throughout the summer from a peak value during the first week of age at 1792 ± 51 mL/day (Parker *et al*., 1990), when milk composition typically increases in ungulates prior to weaning (Oftedal, 1984).

Chan-McLeod *et al*. (1994), in a study with captive caribou and reindeer, found that energy intake increased protein deposition in lactating animals while fat deposition increased in non-lactating females. They also showed that the levels of dry matter, fat, and energy in milk were not affected by maternal energy or protein intake, maternal body condition, or calf age. Lactose content of the milk was correlated with maternal energy intake. During the 12-week experimental period (May to August), body mass changed dramatically between individual lactating animals, ranging from a net loss of 6.4 kg to a net gain of 31.1 kg (Lavigueur & Barrette, 1992; Chan-McLeod *et al*., 1994). At the end of the 12-week study, all caribou gained body weight, while two lactating reindeer showed net losses of body mass and one non-lactating reindeer gained only 2.2 kg.

Using captive woodland caribou in another study, Lavigueur and Barrette (1992) observed that growth rates of woodland caribou from birth to 45 days were positively correlated with suckling rate during the first 35 days. They also noted that growth rates of the young caribou were positively correlated with time spent feeding on pelleted ration and on hay from 46 to 100 days. On the other hand, caribou are not milked for human consumption and thereby make a minimal contribution to human nutrition and health in contrast to the reindeer of northern Scandinavia and Siberia.

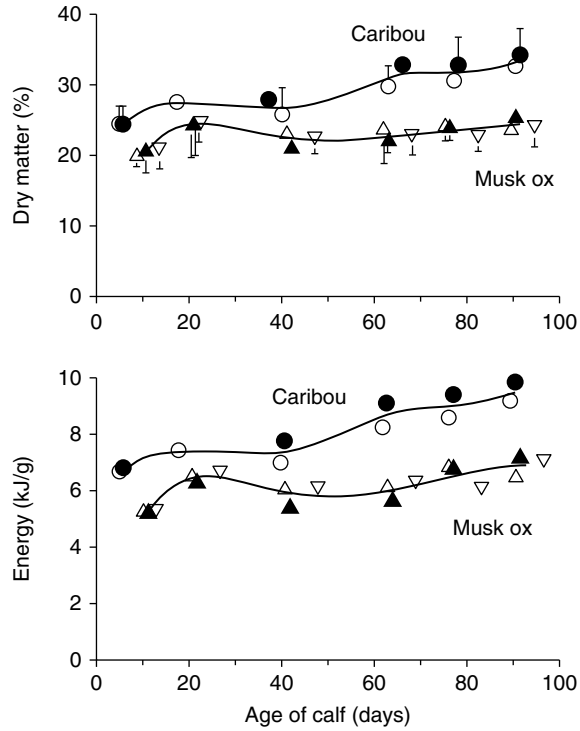


Figure 30.2. Dry matter and energy content of milk from caribou and musk ox during the 100 days following parturition. Adapted from Parker *et al*. (1990) and reproduced from Park & Haenlein (2006), with permission of Blackwell Publishing.

30.3.3 Musk ox

Musk ox (*Ovibos moschatus*) historically ranged across northern Alaska, Canada, and Greenland (Groves, 1992). History reveals that musk ox was extinct in Alaska by the mid 1800s, possibly due to hunting by native people, explorers, and whalers. In the early 1900s, the worldwide musk oxen population was estimated as few as 5000 animals which were considered to be in danger of extinction (Groves, 1992). Beginning in 1917, the Canadian government enforced the protection of the species from hunting and made efforts to save musk oxen (Burch, 1977). Some animals were translocated from Greenland to Alaska and held in captivity in Fairbanks for feeding, growth, and breeding studies until 1935–36, then released into the wild on Nunivak Island (Klein, 1988).

Table 30.4. Gross composition of musk ox milk (mean values).

	Study 1*	Study 2†
Total solids (%)	27.1	–
Water (%)	72.9	–
Fat (%)	10.9	9.45 (range 6.18–12.88)
Solids-not-fat (%)	16.2	–
Ash (%)	1.20	–
Protein (%) (N × 6.38)	11.9	7.33 (range 5.91–9.00)
Lactose (%)	2.1	4.35 (range 3.44–5.03)
Specific gravity	1.023	–
pH	5.4	6.39 (range 6.25–6.54)
Osmolarity (mmol/kg)	–	313.0 (range 293–337)
Sodium (mmol/L)	–	39.1 (range 31–57)
Potassium (mmol/L)	–	34.5 (range 30–40)
Chloride (mmol/L)	–	19.5 (range 14–30)
Urea (mmol/L)	–	25.0 (range 17–31)
Creatinine (μmol/L)	–	197.6 (range 28–354)
Volume (mL/4 h)	–	161 (range 94–280)

*Based on data from Baker *et al*. (1970); average values of five animals.

†Based on data from Chaplin & Follensbee (1991); hand-milked, average values of two musk oxen.

White *et al*. (1989) reported that musk oxen and caribou live in similar arctic environments, and eat similar forages, although their lactational strategies differ in length. Healthy musk oxen continue to nurse their young throughout the rutting period, until December to February, and they may lactate throughout the winter in the field.

The musk ox is an arctic mammal that belongs to the subfamily Caprinae, as do goats and sheep. Musk oxen comprise three recognized subspecies: *Ovibos moschatus moschatus*, *Ovibos moschatus niphoecus* and *Ovibos moschatus wardi*. Musk oxen in Alaska are descendants of the transplanted animals from Greenland, i.e. *O. m. wardi*. A comparative study of allozyme electrophoresis has shown that little genetic variation exists within and between populations of Alaskan and Greenlandic musk oxen (Fleischman, 1986).

Tables 30.1 and 30.4 show the gross composition of musk ox milk, which contains much higher total solids than cow milk with some variations between reports. The higher fat and protein content of musk ox milk is a common characteristic of the milk of arctic and sub-arctic species. Total solids, solids-not-fat, and lactose content significantly increased from day 1 to 3 months of lactation (Table 30.4). Musk oxen have the highest milk production at 3 weeks after parturition, and production remains high for about 1 month, then tapers off gradually (White *et al*., 1989).

Musk ox calves fed milk replacer showed approximately 25% slower growth rates (347 g/day) compared with calves raised naturally suckling their dam (423 g/day) (Chaplin & Follensbee, 1991). Diarrhea was observed as a common problem for those fed milk replacer. Frisby *et al*. (1984) reported the compositional ranges of musk ox milk as milk fat 11.0–15.5%, protein 5.3–15.6%, and lactose 2.9–3.6%, indicating that there are variations in the gross composition of musk oxen milk compared with those values in Tables 30.1 and 30.4.

Concerning the fatty acid composition of musk ox milk, approximately 38% of the total fatty acids is oleic acid, with only trace amounts of butyric acid (Baker *et al*., 1970). Short- and medium-chain fatty acids (C4–C14) of musk ox milk ranged from 9.8 to 17.4%, which is significantly lower than in bovine or caprine milk. Compared with other arctic species, musk ox milk also contains lower levels of long-chain fatty acids (above C18).

30.3.4 Llama milk

The value of South American camelids has been re-recognized in recent decades, and efforts to restore their populations have been made (Sell, 1993; Fowler, 1998). Llama (*Lama glama*) is one of the four main species of New World camelids (Sell, 1993; Fowler, 1998). The llama was domesticated in the Andean puna (elevation 4000–4900 m), probably around Lake Titicaca at about 4000–5000 BC.

Following their domestication, llama herding economies spread beyond the limits of the puna and became a major component in the economy of the Andeans (Fowler, 1998). The Incas depended on llama (and alpaca) for food, fuel, fibers, transport of commodities, and religious rituals (Bastinza, 1979; Fowler, 1998).

In recent decades, llamas have gained increasing interest in the USA and Canada, and the llama industry in these countries continuous to grow. The number of registered owned animals increased from about 70 000–75 000 in 1994 (Morin *et al*., 1995) to about 155 000 in 2003 (International Llama Registry, 2003).

30.3.4.1 Milk yield

The llama has a milk secretory system consisting of four mammary glands, similar in structure to the cow, with four teats each having two streak canals, which enter into separate teat and gland cisterns (Rosenberg, 2006). Milk is collected from variable numbers and sizes of milk ducts from the gland and emptied into the gland cistern. The amount of milk produced daily by individual llamas varies significantly and ranges from 16 to 413 mL per animal, with a median of 62 mL per animal (Morin *et al*., 1995).

30.3.4.2 Milk composition

Llama milk contains an average of 13.1% total solids, 6.5% lactose, 3.4% protein, and 2.7% fat (Morin *et al*., 1995). The energy content of llama milk varies between 50.0 and 95.8 kcal/100 g with an average of 70.0 kcal/100 g, which is lower than that of bovine (85.2 kcal/100 g), caprine (103.6 kcal/100 g) and ovine (155.6 kcal/100 g) milk. Llama milk has a density of 1.033 g/mL, a milk fat density of 0.935 g/mL, and pH of 6.52 at 20°C. Riek and Gerken (2006) reported mean concentrations of the major milk components across the lactation period as 4.70% fat, 4.23% protein, 5.93% lactose, 15.61% dry matter, and 22.62 mg/dL of milk urea nitrogen (Fig. 30.3). All constituents were affected by stage of lactation for these lactating llamas under controlled stable conditions during the first 27 weeks of lactation.

A recent study (Schoos *et al*., 2008) on the fatty acid composition of llama milk showed that the proportions of saturated fatty acids (C4–C10), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids in llama milk fat were comparable to the values in cow milk. The predominant fatty acids in llama milk were C16:0, C18:0, C14:0, and C18:1. The milk also contained *trans* fatty acids at 3 g/100 g total fatty acids (mainly C18:1 *trans*-11), and a small quantity of conjugated linoleic acid (0.4 g/100 g total fatty acids).

Llama milk proteins contain significant proportions of phosphorus, i.e., 0.36, 0.45, 0.30, and 0.15% for α-, β- and γ-casein

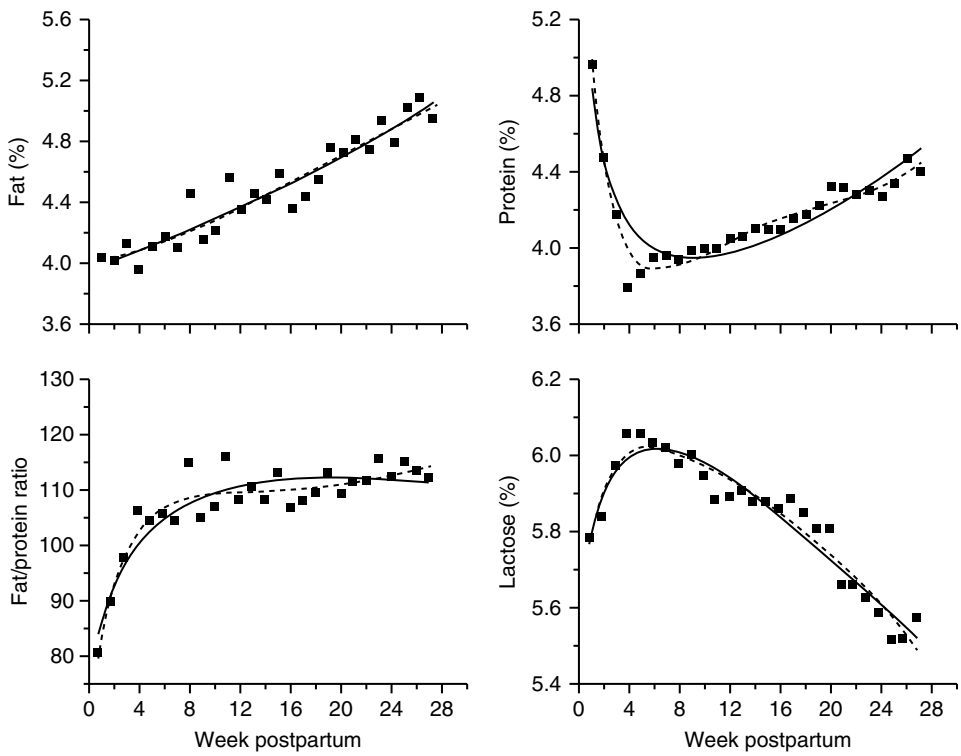


Figure 30.3. Changes in fat, protein, and lactose contents and fat/protein ratio of llama milk during the 27-week lactation period. Reproduced from Riek & Gerken (2006), with permission of Elsevier.

and the proteose peptone fraction, respectively. However, llama milk does not contain β-lactoglobulin (Fernandez & Oliver, 1988; Morin *et al*., 1995). Carbohydrates in llama milk are associated with α-lactalbumin, proteose peptone fractions, immunoglobulins, α-casein, and β-casein. The distribution of sialic acid among protein fractions of llama milk is 84.4% in the milk whey fraction and 15.6% in casein (Fernandez & Oliver, 1988; Rosenberg, 2006).

The mineral content of llama milk is different from human and bovine milk, in which potassium is the most abundant mineral, whereas in llama milk calcium is the main mineral, followed by phosphorus and potassium (Flynn & Cashman, 1997). The calcium content of llama milk is higher (1310–2210 mg/kg) than that in human, cow, and goat milk (280, 1120 and 1400 mg/kg, respectively) but similar to that in camel milk. The sodium concentration in llama milk (193–413 mg/kg) is lower than that in cow milk (530 mg/kg) but higher than that in human milk (180 mg/kg) (Morin *et al*., 1995).

Zinc is the most abundant trace element in llama milk, similar to milk of other species (Anderson, 1992; Rosenberg, 2006). The mean zinc content in llama milk (about 4.2 mg/kg) is higher than that in human milk (1.2 mg/kg) but similar to that in cow milk (3.9 mg/kg) and camel milk (4.0–5.0 mg/kg). Llama milk contains 0.278 mg/kg barium, which is higher than that in cow milk (188 mg/kg). The mean copper concentration in llama milk (0.109 mg/kg) appears to be lower than that in mare (0.155 mg/kg), human (0.250–0.314 mg/kg) or guinea-pig (0.500 mg/kg) milk. Llama milk contains relatively low copper concentrations, consistent with low blood serum copper concentrations in this species compared with other domestic animals. Compared with the iron content in camel milk (1.3–2.5 mg/kg), llama milk has a lower mean level of iron (0.65 mg/kg), comparable to that in cow milk (0.50 mg/kg) but higher than that in human milk (0.3 mg/kg).

30.3.5 Alpaca

The alpaca is one of the two species of domestic South American camelids adapted to live at high altitudes, over 3800 m above sea level (Parraguez *et al*., 2003). There are four species of South American camelids: vicuña, guanaco, llama, and alpaca (Burton *et al*., 2003). The vicuña and guanaco are wild species. The vicuña is native to the altiplano regions of Chile, Bolivia, and Peru. The guanaco is native to the Patagonia regions of southern Chile and Argentina. Llamas and alpacas are the two domesticated species in this camelid family (Fowler, 1998).

Alpacas are an essential source of income and provide meat and wool for the native people who live in the high altiplano regions, but neither alpaca nor llama are milked for human consumption. This camelid is very important for the village economy in Chile, Bolivia, Peru, Ecuador, and Argentina (Burton *et al*., 2003). Alpaca reared in these countries have a conception rate of 50% or lower and a 20% mortality rate of the young (called "crias"). Nutritional inadequacies, infectious diseases, and changes in the environment may cause these reproductive problems (Raggi *et al*., 1994).

Studying the composition of alpaca colostrum at 48 hours after birth for the Andean high plateau and Patagonia populations (Table 30.5), Parraguez *et al*. (2003) reported that the two breeds had 21 and 19% total solids, 9.8 and 9.2% protein, 4.8 and 2.7% fat, 4.4 and 5.3% lactose, and 1.6 and 1.8% ash, respectively. The colostrum of the Andean altiplano breed had higher dry matter, protein and fat, but lower lactose than the Patagonian breed. A similar trend was

Table 30.5. Comparison of gross compositions of alpaca colostrum and mature milk from two regions of Chile.

	Dry matter	Protein	Fat*	Lactose*	Ash
Colostrum					
AHP†	20.66 ± 1.3	9.84 ± 0.6	4.80 ± 1.2	4.41 ± 0.1	1.63 ± 0.0
Patagonia‡	19.06 ± 0.5	9.24 ± 0.5	2.71 ± 0.6	5.33 ± 0.1	1.78 ± 0.1
Mature milk					
AHP†	16.8 ± 0.7	6.9 ± 0.3	3.8 ± 0.6	4.4 ± 0.5	1.7 ± 0.3
Patagonia‡	15.8 ± 0.6	6.5 ± 0.3	2.6 ± 0.5	5.2 ± 0.5	1.4 ± 0.1

*Differences in fat and lactose contents of both colostrum and mature milk between the two regions are significant ($P < 0.05$).

†Andean high plateau region (4400 m above sea level); 24 alpacas tested.

‡Patagonia region (12 m above sea level); 18 alpacas tested.

Source: based on data from Parraguez *et al*. (2003) and reproduced from Park & Haenlein (2006), with permission of Blackwell Publishing.

observed for the major constituents of alpaca mature milk from 1 to 5 months of lactation (Table 30.5). The higher milk fat content of Andean high plateau alpaca compared with the lower-altitude Patagonian alpaca suggests that the animals at higher altitude may require more energy for body maintenance compared with those at lower altitude. The differences in fat and lactose composition between the two regions could also be explained by pasture composition and availability as well as the grazing behavior of the animals.

The main immunoglobulin of colostrum found in alpaca crias is IgG. Bravo *et al*. (1997) showed that the mean IgG concentrations were similar in llama (2370 mg/dL) and alpaca (2340 mg/dL) crias, and not different between male and females. Llama and alpaca crias are born agammaglobulinemic, with IgG concentrations increasing after suckling.

30.3.6 Moose

Studies on domesticated moose (*Alces alces*) have been very limited. Moose milk has the consistency of thick cream and the odor of fresh bovine milk. Moose milk contains less lactose than milk of elk, caribou, or most other wild or minor ruminant species, as shown in Table 30.1 (Park, 2006). In a study with singleton moose calves (*Alces alces gigas*) during the first 4 months after birth, per cent milk composition at peak output was 20.5 ± 1.5 total solids, 7.9 ± 1.5 fat, 7.2 ± 0.4 protein, 1.4 ± 0.1 ash, and 3.7 ± 0.2 lactose, indicating that gross composition of moose milk at peak lactation is lower than in other stages of lactation. Chalyshev and Badlo (2002) showed that initial increases in basic nutrient and fat contents in taiga moose milk during the peak lactation in June (1–25 days) is concurrent with the availability of high-quality forage. They also observed that mineral concentrations in moose milk gradually decreased during 26–100 days of lactation.

There is no marked difference in mineral composition between moose milk and other arctic deer milk, while the potassium content is lower than in other milks, except human milk (Lauer & Baker, 1969; Cook *et al*., 1970a). With regard to fatty acid composition, moose milk has about 53% saturated fatty acids, which is less than the saturated fatty acid content of the milk of most ruminants (Ling *et al*., 1961; Cook *et al*., 1970a). In a study of 21 Alaskan moose at the Kenai Moose Research Center, Soldotna, Alaska, Franzmann *et al*. (1976) reported that Al, Fe, Se, and Zn contents in moose milk were higher than in cow milk by factors of 1.6–290. Ca and Mg levels in hair were lower than those in milk of moose, and were subject to lactational stress such as feed availability and metabolic disease status.

In studying feeding efficiency, Shochat and Robbins (1997) found that maternally raised singleton moose calves had greater milk intake than bottle-raised calves. Although the weight gains of bottle-raised moose calves generally increased as they began consuming significant quantities of forage, poor early growth rates relative to maternally raised calves (790 ± 120 g/day) confirmed the inadequacy of bottle-feeding protocols. In addition, high mortality rates of bottle-raised neonatal moose are frequently associated with improper nutritional management and poor husbandry techniques. Moose calves raised in captivity commonly show dietary-induced diarrhea caused by inappropriate milk replacers. Calves are often underfed to avoid diarrhea, causing poor growth during the first 4 weeks when the young are usually entirely dependent on mother's milk for their growth and survival (Shochat & Robbins, 1997).

Moose milk is commercially farmed in Russia (Fig. 30.4, see also Plate 30.1): one sanatorium, the Ivan Susanin Sanatorium, even serves moose milk to residents in the belief that it helps them recover from disease or manage chronic illness more effectively (Grocott, 1994). Some Russian researchers have recommended that moose milk could be used for the prevention of gastroenterological diseases in children, due to its lysozyme activity



Figure 30.4. A milkmaid at the Kostroma Moose Farm in Kostroma Oblast, Russia, prepares to milk a moose. From http://ca.wikipedia.org/wiki/Fitxer:Milkmaid-and-Moose-Cow-hp4080.jpg. Courtesy of Dr. Alexander Minaev. For a color version of this figure, see Plate 30.1.

(Dorofeichuk *et al*., 1987). The Elk House in Sweden has three milk-producing moose, whose milk yields roughly 300 kg of cheese per year, and the cheese sells for about $1000 per kilogram (Johansson, 2007).

In a comparative study on the fatty acid patterns of moose, camel, cow, and human milk, Dreiucker and Vetter (2011) reported that the animal milks contained up to eight *iso*-fatty acids (iFA) ranging from C13 to C22 as well as four *anteiso*-fatty acids (aFA) with odd chain length (C13–C19) (Fig. 30.5). The higher diversity of iFAs compensated for this, so that the iFA/aFA ratio was similar in all samples and slightly above 1 (Fig. 30.5a). The *trans*-MUFA pattern was dominated by *trans*-11 C18:1, which contributed between 30 and 50% to the *trans*-MUFA content (Fig. 30.5b) and which could be as high as 2.7% (moose) and 2.6% (cow). The most remarkable fact in the group of *cis*-18:1 isomers was the relatively low content of C18:1 in human milk and higher proportions in moose and cow milk.

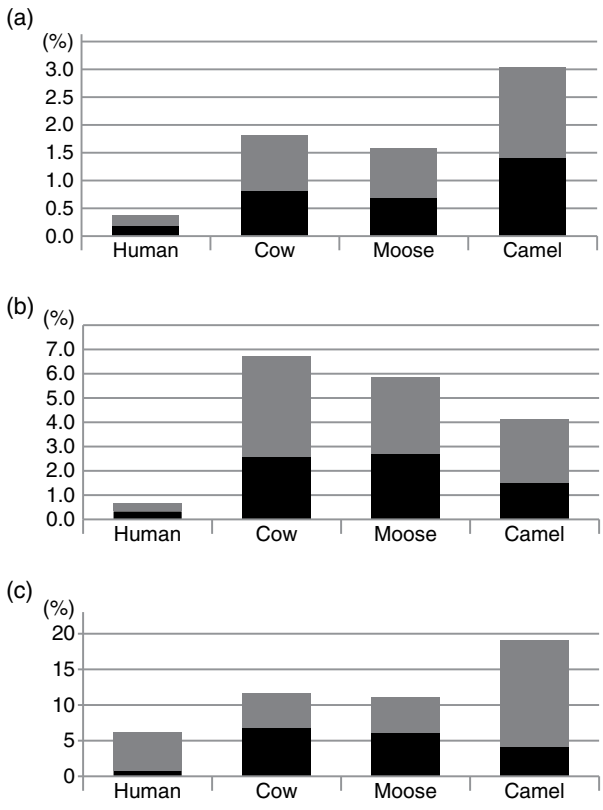


Figure 30.5. Content (g/100 g fatty acids) of (a) *iso*- (gray) and *anteiso*-fatty acids (black), (b) *trans*-monounsaturated fatty acid (MUFA) other than 18:1(11tr) (gray) and 18:1(11tr) (black), as well as (c) *cis*-MUFA without 18:1(9) (gray) and *trans*-MUFA (black) in human, cow, moose, and camel milk. Reproduced from Dreiucker & Vetter (2011), with permission of Elsevier.

30.3.7 Elk

Very limited reports are available on production and composition of elk milk. Growth rates of hand-raised elk are lower than those of dam-raised elk. For hand-raising wild ungulates, evaporated milk fed *ad libitum* appeared to be an effective and practical approach. Robbins *et al*. (1981), studying the milk composition of a captive elk (*Cervus elaphus nelsoni*), have shown that it contained 19.8% dry matter, 6.2% protein, 7.5% fat, 4.1% lactose, and 1.1% ash during the first 3 months of lactation. The calf had its peak milk intake at 21 days, then milk intake gradually decreased during the following 2 months. A significant dry-feed intake was initiated prior to the decline in milk intake, indicating that meeting the calf's nutrient requirements would be increasingly important after 40 days. Wild *et al*. (1994) found that growth rates of hand-raised elk were lower than those of dam-raised elk.

Vasilenko *et al*. (2002) conducted a study on the milk productivity of five domesticated elk females during 17 lactation periods over 4 years at the Pechoro-Ilych State Nature Reserve, Russia. On average, the lactation period of elk cows was 105 days, peak daily milk production (3.4–7.6 L per elk cow) occurred at 20 days after birth, and milk production declined by 50–60 days after birth due to estrous activity.

Fat composition of elk milk is similar to that of cow milk, containing high levels of palmitic, stearic, oleic, and myristic acids along with smaller amounts of short-chain fatty acids. Smith *et al*. (1997) reported that early development of cervids is attributable to juvenile survival and lifetime reproductive success. Male calves of free-ranging elk are usually born late and weigh more than females, and supplemental feeding has revealed little effect on birthweight of elk calves. Nutritional benefits of winter feeding on maternal condition entering late gestation may improve milk yields of dams, benefiting the weight gains of the first week of life for elk neonates.

30.3.8 Mithun

The mithun (*Bos frontalis*) is a domesticated bovine species mainly found in the hill regions of India, Myanmar, Bhutan, and Bangladesh (Nath & Verma, 2000). The mithun plays an important role in the economic well-being and social and cultural life of the rural people in these countries. Hybrids of mithun and cattle are used as dairy animals in parts of north-eastern India and Bhutan (Indian Council of Agricultural Research, 2010).

Mithun milk contains higher total fat and protein than cow milk, and the total protein and fat content of individual mithun at late lactation have been reported as 6.78 g/100 g and 10.3 g/100 g, respectively (Mech *et al*., 2008). This fat content is unusually high: the mithun is not an arctic animal, but its habitat is at an elevation of 1000–3000 m above sea level. The high levels of fat and protein in mithun milk may be attributable to the unique genetic composition and the low milk yield of the species (Mech *et al*., 2008). Lactose content of mithun milk (4.44 g/100 g) is similar to that of cow and goat milk, while the ash content of mithun milk (0.9 g/100 g) is higher than that in cow and goat milk (Park, 2006; Mech *et al*., 2008).

30.3.9 Other minor species

Interesting minor species for study of milk composition include pinnipeds, elephant and polar bear. These species are not domesticated but their milk can be accessible. Pinnipeds represent a group of marine mammals that includes the northern elephant seal, antarctic fur seal, California sea lion, and Australian sea lion.

30.3.9.1 Pinniped

Pinniped milk has a distinctly different gross composition compared with other mammalian species, being rich in fat, as high as 60% in phocids (seals) and about 20–35% in otariids (sea lions), with about 10–12% protein, the highest among mammals (Oftedal *et al*., 1987; Davis *et al*., 1995).

The mean milk composition of sub-antarctic fur seals is 42.8 ± 5.7% lipid, 12.1 ± 1.5% protein, and 42.6 ± 7.3% water (Table 30.6). Georges *et al*. (2001) found that the fur seals breeding on Amsterdam Island produced one of the richest milks ever reported in otariids (20.4 ± 2.9 kJ/g),

Table 30.6. Mean gross chemical composition of milk in sub-antarctic fur seals on Amsterdam Island, collected during the entire pup-rearing period.

	Mean	SD	*N*	Range
Lipid (%)	42.8	5.7	98	24.7–56.4
Protein (%)	12.1	1.5	83	9.2–15.4
Water (%)	42.6	7.3	83	26.2–64.2
Total mass (%)	98.1	0.9	83	95.5–99.9
Gross energy (kJ/g)	20.4	2.9	83	12.2–27.6

Note: mean values are means of individual average values, regardless of the time mothers spent fasting ashore before sampling occurred.

Source: reproduced from Georges *et al*. (2001), with permission of Chicago University Press.

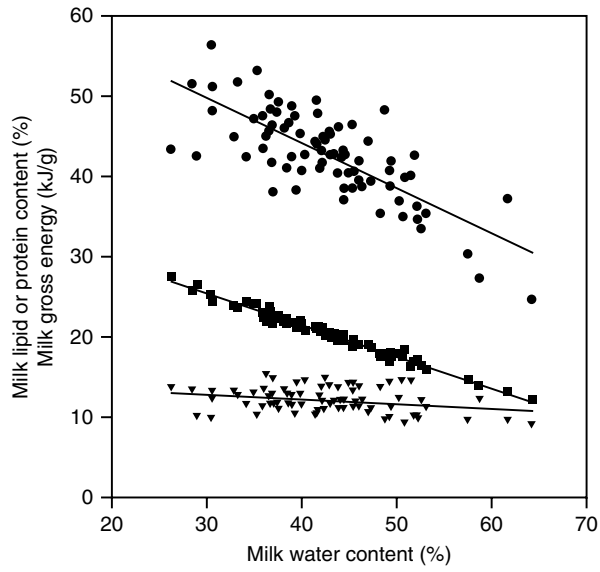


Figure 30.6. Relationships between water (%) and lipid (%; circles), protein (%; triangles), and gross energy (kJ/g; squares) in sub-antarctic fur seal milk at Amsterdam Island. Reproduced from Georges *et al.* (2001), with permission of Chicago University Press.

with lipid content contributing 85% of total gross energy. On average, the sum of measured protein, lipid, and water accounted for 98.1 ± 0.9% of the milk mass. There were negative relationships (Fig. 30.6) between water content and lipid content [lipid (%) = 66.59 – 0.56 × water (%); $r^2 = 0.571$, $N = 83$, $P < 0.001$], protein content [protein (%) = 14.54 – 0.058 × water (%); $r^2 = 0.08$, $N = 83$, $P < 0.009$], and gross energy [gross energy (kJ/g) = 37.02 – 0.389 × water (%); $r^2 = 0.976$, $N = 83$, $P < 0.001$] (Georges *et al*., 2001).

There was a commonality in milk amino acid patterns in pinniped milk relative to other species, despite wide variation in total amino acid concentrations (Davis *et al*., 1995). The most abundant amino acids in pinniped milk are glutamate, proline, and leucine.

Lactoferrin is an iron-binding glycoprotein found in different biological fluids of mammals and in neutrophils. Conesa *et al*. (2008) purified lactoferrin using ion-exchange chromatography (SP-Sepharose) and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) from milk of several species: sheep (*Ovis aries*), goat (*Capra hircus*), camel (*Camelus bactrianus*), alpaca (*Lama pacos*), elephant (*Elephas maximus*), and gray seal (*Halichoerus grypus*), as well as human (Fig. 30.7). The thermal stability of the purified lactoferrins,

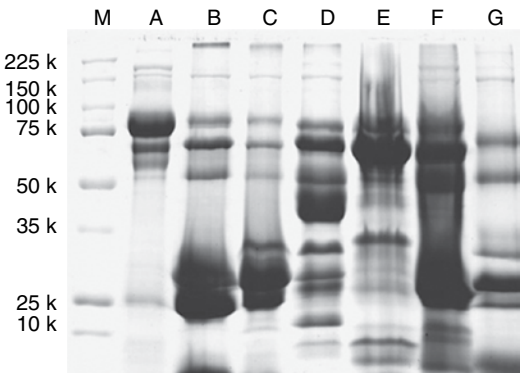


Figure 30.7. Isolation of lactoferrin (80 kDa) from milks of different species by SDS-PAGE: A, human; B, sheep; C, goat; D, camel; E, alpaca; F, elephant; G, gray seal. M, molecular weight marker. Reproduced from Conesa *et al.* (2008), with permission of Elsevier.

in their native and iron-saturated forms, was also investigated by differential scanning calorimetry. Lactoferrin was identified by SDS-PAGE in all milks as a band of 80 kDa apart from the seal milk (Fig. 30.7). The chromatographic profile followed a similar pattern, with only one peak in the elution step with 1 mol/L NaCl and a main band of 80 kDa in the SDS-PAGE analysis. In the case of the gray seal milk, no protein was detected at 280 nm in the elution step with 1 mol/L NaCl and SDS-PAGE did not reveal the presence of any protein with a molecular mass of 80 kDa. Camel milk lactoferrin showed the most active antimicrobial activity against *Escherichia coli* O157:H7, whereas alpaca and human lactoferrins were the least active.

30.3.9.2 Polar bear

Polar bear milk is creamy white and has a strong fishy odor, whereas grizzly bear milk is pale yellow with a consistency of thick cream and the odor of fresh cow milk (Cook *et al.*, 1970b). As the stage of lactation advances, total solids content of polar bear milk decreases slightly, but is considerably higher than in milk of other land mammals, ranging from 43 to 47%. Fat, solids-not-fat, and protein are extremely high, while lactose content is quite low compared with cow milk, but closer to marine mammals. Grizzly bear milk fat has a lower palmitoleic and higher linoleic acid content than does polar bear milk (Cook *et al.*, 1970b).

In studying hexose and sialic acid content of seven polar bear milk samples, Urashima *et al.* (2000) found that hexose and sialic acid levels in polar bear milk ranged from 0.30 to 3.04 and from 0.18 to 0.43 g/dL, respectively. They also reported that the respective mean (±SD) values for hexose and sialic acid of the seven samples were 1.21 ± 1.13 and 0.32 ± 0.09 g/dL. These authors showed that lactose was present only in small amounts. Some of the milk oligosaccharides of the polar bear had α-Gal epitopes similar to some oligosaccharides in milk from the Ezo brown bear and the Japanese black bear. Some milk oligosaccharides had human blood group A antigens as well as B antigens; these were different from the oligosaccharides in Ezo brown and Japanese black bears.

30.3.9.3 Elephant

In a study of the milk of an Asian elephant (*Elephas maximus*) at 11 days after birth, Uemura *et al.* (2006) found that the milk contained 91 g/L of hexose and 3 g/L of sialic acid. The dominant saccharide in this milk sample was lactose, but it also contained isoglobotriose, i.e., Glc(α1→3)Gal(β1→4)Glc, as well as a variety of sialyloligosaccharides. The structures of the sialyloligosaccharides were determined to be those of 3′-sialyllactose, 6′-sialyllactose, monofucosyl monosialyl lactose, i.e., Neu5Ac(α2→3)Gal(β1→4)[Fuc(α1→3)]Glc, sialyllacto-*N*-neotetraose c (LSTc), galactosyl monosialyl lacto-*N*-neohexaose, galactosyl monofucosyl monosialyl lacto-*N*-neohexaose, and three novel oligosaccharides. The oligosaccharide in fraction Em 1-2-11 was characterized by comparison of its proton NMR spectrum (Fig. 30.8, chemical shifts) with that of Em 1-2-7 (LSTc). The spectrum had the anomeric signals of α-Glc, β-Glc, β(1→3)-linked GlcNAc, and two of β(1→4)-linked Gal at δ 5.219, 4.663, 4.726, 4.455, and 4.438, respectively. The oligosaccharide contained an additional Gal(β1→4)GlcNAc(β1→3) unit, namely it had a *para*-lacto-*N*-neohexaose unit, i.e., Gal(β1→4)GlcNAc(β1→3)Gal(β1→4)GlcNAc(β1→3)Gal(β1→4)Glc.

Osthoff *et al.* (2007) observed the dynamic changes in nutrients of the milk from three free-ranging African elephant (*Loxodonta africana africana*) cows during lactation. The respective nutrient contents over 12, 14, and 18 months of lactation were 47.3, 52.0, and 68.6 g protein; 60.7, 87.4, and 170.8 g fat; 1.6, 2.1, and 0.5 g lactose, and 20.9, 21.5, and 8.6 g oligosaccharides per kilogram milk. The protein fraction consisted of 18.0, 31.7, and 45.9 g caseins per kilogram milk and 29.3, 20.3, and 22.7 g whey proteins per kilogram milk, respectively (Osthoff *et al.*, 2007). Electrophoresis and identification of protein bands showed that polymorphs of one whey protein may be present in elephant milk similar to polymorphs of α-lactalbumin found in cow milk.

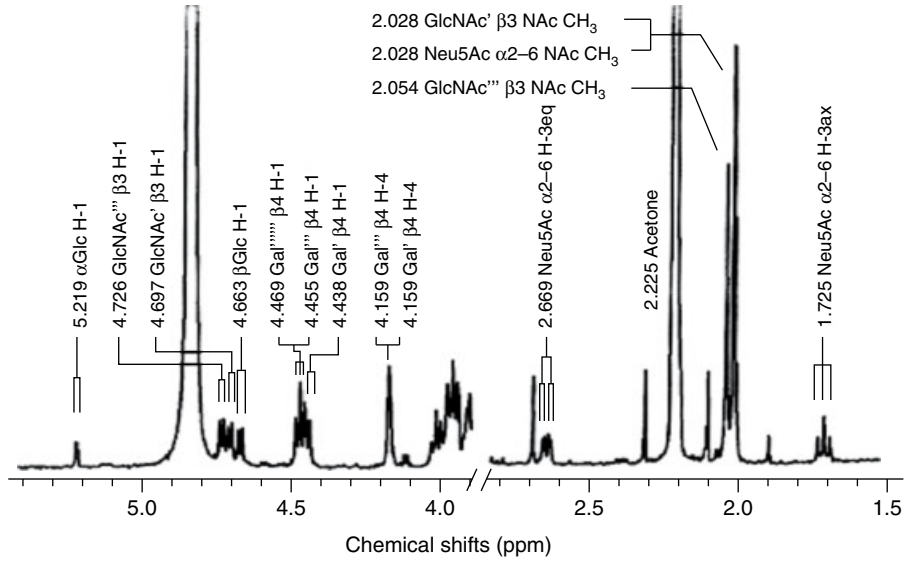


Figure 30.8. Oligosaccharides (600 MHz ^{1}H-NMR spectrum of Em 1-2-11) isolated from Asian elephant milk. The spectrum was obtained in D2O at 600 MHz with a Varian INOVA 600 spectrometer operated at a probe temperature of 293.1 K. Reproduced from Uemura *et al.* (2006), with permission of Elsevier.

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31
Human Milk

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31.1 INTRODUCTION

Milk has been described as the most perfect food in nature. Milk is balanced for most nutrients and often has a high caloric value. It can meet the nutritional requirements of the newborn during its early critical period of body development, and provides essential nutrition for normal growth, until the newborn is able to consume and digest solid foods (Park & Haenlein, 2006). All mammalian young are completely dependent on mother's milk until they begin to feed on their own and are weaned weeks after parturition. Although qualitative nutritional characteristics of a species milk may be similar to those of other mammals, the quantities of nutrients in milk differ considerably between species (Widdowson, 1970).

Human milk from healthy and well-nourished mothers is preferred for all newborn infants. Since breastfeeding, both exclusively and partially, confers health benefits to infants and mothers, the World Health Organization recommends that all babies should be exclusively breast-fed for the first 6 months after birth and continued breastfeeding up to 2 years of age and beyond (WHO/UNICEF, 2005). No other foods may be provided, including no solid foods nor infant formula nor water for babies during the first 6 months after birth (World Health Organization, 2002). To translate this recommendation into practice provides a challenge for all health professionals working to improve the health of all infants. Very few infants in the developed world are exclusively fed human milk for 6 months (Morgan, 2006). The rate in the UK in 2000 was 2% based on a nationally representative sample (Hamlyn *et al*., 2002). Moreover, this recommendation may not be advantageous for subgroups in the population. Thus low-birthweight infants (weight <2500 g at birth) and preterm infants (born before 37 weeks of gestation) have specific nutritional needs that are probably not met by exclusive human milk feeding for even 4 months post term (Foote & Marriott, 2003).

Feeding human milk has been shown to have many benefits for infants, including a reduced risk of neonatal necrotizing enterocolitis, gastroenteritis, respiratory infection and immunologically based disease, and improved later cognitive development (Wagner *et al*., 1996; Schanler & Atkinson, 1999). Besides macronutrients, energy and micronutrients, human milk contains a wide range of biologically active substances, including immunoglobulins, hormones, growth factors, and at least 60 enzymes (Morgan, 2006). These bioactive substances in human milk play important roles in the non-nutritional effects of human milk on the development of the infant (Lönnerdal, 2003). The clinical findings indicate that human milk may exert protective effects on infants during neonatal maturation. This chapter addresses the major issues involved in current concepts, composition, production, and practices of human milk in relation to human nutrition and health.

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31.2 HUMAN MILK FEEDING AND ITS PRACTICE

Human breast milk is a complex living nutritional fluid. Its provision is an elaborate process in all mammals, with changes in milk composition and interactions between parent and young beyond the straightforward nutritional function (Lefevre *et al*., 2010). First milk produced, or colostrum (from birth to 5 days after birth), is a low-fat, high-protein fluid with a high content of immunoprotective compounds. The milk produced from 5 to 14 days after birth is termed "transitional milk" and its composition is highly variable. Typically, by 15 days after birth, mature human milk is produced and its composition is relatively stable until weaning.

The volume of mother's milk and its energy and fat content increase over the first months of lactation as growth proceeds and appetites develop. After breastfeeding of the infant is established, the number of feedings may be reduced to six to eight over 24 hours, although there is wide variation in infants' nutritional demands as well as mothers' volume of milk production. Exclusive feeding of mother's milk with no other foods or drinks for 6 months from birth is recommended for maintaining a high milk output and sustaining health advantages to the infant. The "Baby friendly" initiative was established in the 1980s by the World Health Organization, with 10 steps to successful breastfeeding as depicted in Table 31.1 (Lawson, 2003).

On the other hand, the majority of women in developed countries are unable or unwilling to continue breastfeeding for 6 months, because some women find this practice unrewarding and not feasible. More than one-third of first-time mothers in maternity units experience problems feeding their infants. Many of these problems (perceived milk insufficiency, painful breasts or nipples, and problems with suckling) can be addressed with lactational support (Morgan, 2006). Women who need to return to work within 6 months of the birth of their child would cease breastfeeding sooner or are less likely to feed initially, compared with those women who do not work. The introduction of infant formula together with solid food introduction invariably reduces milk output. In addition, convenience and economic considerations influence the choice of many mothers' need to work outside their homes.

Several studies throughout history have shown that infants who are not breast-fed have a greater incidence of disease relative to those who are breast-fed (Newburg, 2009). A study in Chicago in the 1920s of more than 20 000 mother–infant dyads reported that infants not breast-fed had sevenfold, twofold, and fourfold greater mortality caused by gastrointestinal, respiratory, and other diseases, respectively (Grulee *et al*., 1934). The protection against infection has been shown to persist into the second and even third years of life and is probably due to a large extent to immunological factors detected in human milk. Specifically, human milk contains an abundant oligosaccharide fraction (HMOs), secretory antibodies (IgA) and multifunctional components (e.g., peptides from partial digestion products of milk proteins, such as lactoferrin, or free fatty acids and monoglycerides from partial digestion of human milk triacylglycerols) (Newburg, 2009; Petherick, 2010). Recently, immune-regulatory proteins have been identified in human milk. Of particular interest are cytokines, which orchestrate the development of the immune system by signaling between immune cells (Garofalo, 2010; Petherick, 2010).

In the last decades breastfeeding has also been studied with respect to its potential effect on longer-term health during childhood and even in adulthood (Kramer, 2010). Protective effects of human breast milk have been reported against obesity (Owen *et al*., 2005a, b), hyperlipidemia (Owen *et al*., 2002), hypertension (Martin *et al*., 2005), insulin resistance and type 2 diabetes (Owen *et al*., 2006), atopic disease (including atopic eczema, asthma, hay

Table 31.1. Ten steps to a baby-friendly environment.

Step 1: Have a written breastfeeding policy that is routinely communicated to all healthcare staff
Step 2: Train all healthcare staff in skills necessary to implement this policy
Step 3: Inform all pregnant women about the benefits and management of breastfeeding
Step 4: Help mothers initiate breastfeeding within half an hour of birth
Step 5: Show mothers how to breastfeed and how to maintain lactation even if they should be separated from their infants
Step 6: Give newborn infants no food or drink other than breast milk, unless medically indicated
Step 7: Practice rooming-in to allow mothers and infants to remain together 24 hours a day
Step 8: Encourage breastfeeding on demand
Step 9: Give no artificial teats or pacifiers or soothers to breast-fed infants
Step 10: Foster the establishment of breastfeeding support groups and refer mothers to them on discharge from hospital

Source: Based on data from World Health Organization (1989) and Lawson (2003).

fever, and positive skin tests) (Gdalevich *et al*., 2001a, b), and even against other rarer diseases with a presumed immunologic basis, such as type 1 diabetes, inflammatory bowel disease, leukemia, and lymphoma (Ip *et al*., 2007). Finally, breastfeeding has also been associated with improved cognitive ability (Anderson *et al*., 1999, Guxens *et al*., 2011).

31.3 PRODUCTION OF HUMAN MILK

Milk constituents are produced by the epithelial cells in the mammary glands via two different secretory processes: synthesis and diffusion. The major compounds such as milk fat, most of the protein components, and lactose are synthesized in the epithelial cells from blood precursors and then released into the lumen of the alveolus (Schmidt, 1971). The remaining milk constituents including water, vitamins and minerals diffuse from the blood and move across the epithelial cells. The major determinants of composition and production volume of human milk are the expenditures of energy, protein and other nutrients by lactating and breastfeeding women.

Well-nourished women who are breastfeeding exclusively produce on average 750 mL/day (Morgan, 2006). If the energy content of milk is 67 kcal/dL, approximately 500 kcal/day is secreted in the milk. There have been estimates of the cost of synthesizing human milk and they range from 25 to 125 kcal/day in the production of 750 mL of milk depending on estimates of the efficiency with which the milk is produced and secreted (Morgan, 2006).

At around 10 days after birth the production of mature human milk is established. During the proceeding days, weeks and months there are further changes in milk composition and in volume (Table 31.2), but these changes are relatively small. Although the mean values for volume are similar over time, there is high individual variation around the mean, and the volume is influenced by the practice of complementary feeding (Table 31.2). There is wide variation between mothers in the rate of transfer of milk during the course of a feed. Therefore, the concept of an average time over which a single feed takes place does not exist. Maternal factors (the milk supply and secretion rate) and infant needs (hunger, suckling pattern) are thought to influence milk flow. The research evidence has shown that milk intake is primarily driven by the infant, whose "demand" for milk regulates milk output (Morgan, 2006).

31.4 COMPOSITION OF HUMAN MILK

31.4.1 General composition

Human milk does not have a constant but a complex and dynamic composition. The compiled nutritional composition of mature human milk can be found in several food composition databases, most of them available online (Food and Agriculture Organization, 2012). A comparison of the major composition of human and bovine milk is presented in Fig. 31.1. Besides nutrients, human milk also contains hormones, growth factors, immunoglobulins, cytokines, enzymes, and other molecules that support both the growth and the passive defenses of the newborn.

Table 31.3 compares the energy and macronutrient composition of human milk with eight other mammalian species. One of the most fascinating facts regarding the quality and quantity of milk compostion is that they determine the rate of growth of the offspring of a specific mammalian species. Protein content of milk differs by as much as 10 times between species (Table 31.3). However, the macronutrient which differs most among the species is fat, which determines to a great extent the overall energy content. Human and cow milk have the lowest concentrations of energy and fat, while the milk of blue whale has 10 times higher fat and six times higher energy content than that of human milk (Widdowson, 1970).

The growth rates of offspring of various mammals depend largely on the compositional differences in the milk (Table 31.3). Among the major constituents, protein, mineral and energy content are highly positively correlated to the growth rate of the young. The consequences of these differences in major milk components are illustrated in

Table 31.2. Mean (range) of milk output (g/day) from women living in developed societies.

	Stage of lactation		
Location	**1 month**	**3 months**	**6 months**
Sweden (exclusive milk feeding)	610 (416–839)	766 (497–1029)	788 (510–1123)
Australia (exclusive milk feeding)	1187 (799–1611)	No data given	1128 (608–1610)
UK (mixed feeding)	740 (480–1059)	784 (280–1114)	493 (135–906)
USA (mixed feeding)	569 (398–989)	523 (242–1000)	436 (147–786)

Source: based on data from Williams (1991).

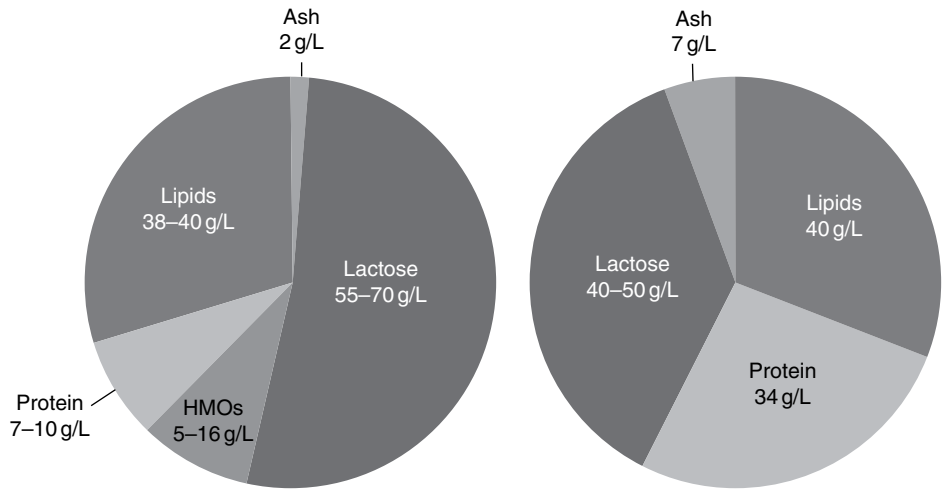


Figure 31.1. Composition of major components of human (left) and bovine (right) milk. Based on data from Coppa *et al.* (1993, 1999), Kunz *et al.* (2000) and Nasirpour *et al.* (2006).

Table 31.3. Average energy and nutrient composition of milk from nine mammals per 100 g milk.

Mammal	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrates (g)
Rat	134	9	9	3
Cat	159	11	11	3
Dog	134	8	9	4
Pig	129	6	9	5
Human	70	1	4	7
Cow	70	3	4	5
Elephant	121	5	9	4
Hippopotamus	205	7	18	2
Blue whale	426	12	40	1

Source: based on data from Widdowson (1970).

the substantial differences in growth rates (Table 31.4). The mean growth rate of the human infant during the milk feeding period is 25 g/day, whereas that of the newborn blue whale is 86 000 g/day. On the other hand, growth is not only dependent on milk composition, but also on the amount of the milk consumed.

Human milk resembles blood in composition, while the commonly used substitute for human milk such as cow milk-based infant formula may be considered a simple nutrient medium (Lucas, 1993). The nutrient composition of human milk may not be uniform, and changes during the course of feeding and during the stage of lactation of lactating women.

31.4.2 Milk protein

Human milk is a low-protein lacteal secretion, with the protein/energy ratio of 13% for colostrum falling to 7% in mature human milk (Table 31.5). The protein content of human milk decreases rapidly during the first month of lactation (14–16 g/L during early lactation, 8–10 g/L at 3–4 months, and 7–8 g/L at 6 months and later). This decrease is mainly due to the diminution in whey protein concentration. These changes result in a whey protein to casein ratio of about 90 : 10 in early lactation, 60 : 40 in mature milk and 50 : 50 in late lactation (Kunz & Lönnerdal, 1992). In contrast, about 80% of bovine milk proteins consist of caseins (Severin & Xia, 2005).

Table 31.4. Birthweight, length of lactation, weight at weaning and growth rate of nine mammals.

Mammal	Mean birthweight (g)	Lactation (days)	Weaning weight (g)	Mean growth rate (g/day)
Mouse pup	2	15	9	0.47
Rat pup	5	21	40	1.7
Kitten	100	35	600	14
Dog pup	100	35	1 200	29
Piglet	1 500	56	18 000	295
Human infant	3 500	180	8 000	25
Calf	35 000	60	70 000	580
Elephant calf	114 000	1460	600 000	335
Blue whale calf	3 000 000	210	21 000 000	86 000

Source: based on data from Widdowson (1970).

Table 31.5. Energy, macronutrient and selected micronutrient content of colostrum, transitional and mature human milk, infant formula and cow milk (per dL).

Nutrient	Colostrum	Transitional human milk (day 10)	Mature human milk	Cow milk	Infant formula* (whey dominated)
Energy (kcal)†	56 (235)	67 (280)	67 (280)	66 (275)	67 (280)
Protein (g)‡	2.0 (13)	1.5 (9)	1.3 (7)	3.3 (20)	1.5 (9)
Fat (g)§	2.6 (41)	3.7 (50)	4.2 (53)	3.8 (51)	3.6 (48)
Carbohydrate (g)¶	6.6 (46)	6.9 (41)	7.0 (40)	4.8 (29)	7.2 (43)
Sodium (mg)	47	30	15	55	16
Calcium (mg)	28	25	35	120	59
Zinc (mg)	0.6	0.3	0.3	0.4	0.6
Iron (mg)	0.1	0.1	<0.1	0.06	0.8
Retinol (μg)	115	85	60	35	75
Vitamin D (μg)	N	N	0.01	0.08	1.0
Vitamin C (mg)	7	6	4	1.8	5.2
Folate (μg)	2	3	5	6	4
Thiamin (mg)	Trace	0.01	0.02	0.04	0.04
Riboflavin (mg)	0.03	0.03	0.03	0.07	0.06
B_{12} (μg)	0.1	Trace	Trace	0.4	0.1

*May contain nucleotides.
†Values in parentheses indicate energy in kJ.
‡Values in parentheses indicate protein/energy ratio (%).
§Values in parentheses indicate fat/energy ratio (%).
¶Values in parentheses indicate carbohydrate/energy ratio (%).
N, significant quantities but no reliable information.
Source: based on data from Morgan (2006).

Human milk also contains a wide variety of proteins with multiple biological activities, including modulation of digestion and utilization of macronutrients and micronutrients, immunomodulatory activities, trophic effects on intestinal mucosa, and hormonal activities (Le Huerou-Luron *et al*., 2010). Recently, the proteomic characterization of specific minor proteins in the human milk casein fraction and whey protein fraction has been reported, unraveling the complexity of the human milk protein fraction (Liao *et al*., 2011a, b). Whey proteins in human milk include α-lactalbumin, sIgA, lactoferrin and lysozymes, but no β-lactoglobulin (Fomon, 1993). The relatively high protein content of infant formulae compared with human milk seems to be associated with a higher body

weight up to age 2 and possibly in latter life (Koletzko *et al*., 2009).

Human milk contains about 25% of total nitrogen as non-protein nitrogen, of which 50% is urea, with small amounts of glucosamines, nucleotides, free amino acids, polyamines, and biologically active peptides. Taurine is one of the free amino acids and a growth modulator, and is at higher concentration in human milk than in cow milk. Recently, some cow milk infant formulae have been supplemented with taurine. A low-birthweight formula available in the UK contains 5.5 mg/dL of taurine (King & Harrison, 2003). Human milk has relatively high levels of carnitine, an amino acid-like substance, which is involved in the oxidation of fatty acids. Infants can synthesize carnitine, but preterm infants and those undergoing catch-up growth may be unable to synthesize it at a sufficiently rapid rate to meet demand and supplementation may be advisable (Morgan, 2006). A low-birthweight formula available in the UK contains 2.0 mg/dL of carnitine (King & Harrison, 2003).

31.4.3 Milk carbohydrates

31.4.3.1 Major carbohydrates

Lactose accounts for 80% of the carbohydrates in human milk and for approximately 40% of its total energy (Morgan, 2006). Lactose in milk is particularly beneficial for infants and adults due to its high solubility, promotion of protective intestinal flora (e.g., *Bifidobacterium bifidus*), reduction in glycemic index in diabetics, enhancement of calcium absorption, reduction in cariogenicity compared with other sugars, and prevention of constipation. Other carbohydrates in milk include monosaccharides, oligosaccharides and protein-bound carbohydrates (Weaver & Prentice, 2003). Lactose content markedly increases during the lactation phase, from 30–50 g/L in colostrum to about 70 g/L in mature milk, around 40% of the energy content in mature human milk.

Human milk oligosaccharides (HMOs) are a third major component of human milk. Human colostrum is reported to contain 20–23 g/L oligosaccharides, and mature human milk to contain 5–16 g/L oligosaccharides (Coppa *et al*., 1993, 1999; Kunz *et al*., 2000). Bovine milk has very low levels of oligosaccharides compared with human milk. Mature bovine milk contains approximately 1.6 g/L in early post-parturition milk (colostrum), decreasing to traceable levels (0.06 g/L) (Fong *et al*., 2011).

31.4.3.2 Human milk oligosaccharides and infant microbiota

Human milk contains abundant, diverse and complex oligosaccharides apparently indigestible by the developing infant and instead targeted to its cognate gastrointestinal microbiota (Zivkovic *et al*., 2011). Mature human milk contains about 5–15 g/L of unbound oligosaccharides in addition to lactose, similar to the protein content, and exceeds the amount of milk lipids. Milk oligosaccharides are both neutral and anionic species, with building blocks of five monosaccharides: D-glucose (Glc), D-galactose (Gal), *N*-acetylglucosamine (GlcNAc), L-Fucose (Fuc), and *N*-acetylneuraminic acid (Neu5Ac, commonly sialic acid) (Fig. 31.2, see also Plate 31.1) (Bode, 2006; Zivkovic *et al*., 2011). Lactose (Galβ1$\rightarrow$4Glc) forms the reducing end of milk oligosaccharides. Gal in lactose can be sialylated in α2$\rightarrow$3 and/or α2$\rightarrow$6 linkages to form 3′-sialyllactose and 6′-sialyllactose, respectively. Lactose can also be fucosylated in α1$\rightarrow$2 and α1$\rightarrow$3 linkages to form 2′-fucosyllactose and 3′-fucosyllactose, respectively. These trisaccharides are called the short-chain milk oligosaccharides. To form more complex milk oligosaccharides, lactose is elongated with up to 25 *N*-acetyllactosamine repeat units (Galβ1$\rightarrow$3/4GlcNAc). Lactose or the polylactosamine backbone can be sialylated in α2$\rightarrow$3 and/or α2$\rightarrow$6 linkages and/or fucosylated in α1$\rightarrow$2, α1$\rightarrow$3, and/or α1$\rightarrow$4 linkages (Fig. 31.3, see also Plate 31.2) (Bode, 2009; Wu *et al*., 2010).

More than 200 HMOs with structural differences related to their size, charge and sequence have been identified (Wu *et al*., 2010, 2011). HMO mixtures are highly complex and can vary widely among and within women. Even in the degree of terminal fucosylation and sialylation, HMOs can vary in the range of 50–70% and 5–15%, respectively. However, the complete picture on this variation has not yet been entirely described (Zivkovic *et al*., 2011).

One factor that appears to be related to differences in HMOs among mothers is the genotype related to fucosyltransferase expression, namely the secretor (Se, expresses fucosyltransferase II) and Lewis (Le, expresses fucosyltransferase III, which catalyzes the formation of α1$\rightarrow$3/4-linked fucose) genes (Newburg, 2009). Four different oligosaccharide milk groups that correspond well to the genetic basis of the Lewis blood group system have been identified (Thurl *et al*., 1997):

1. Lewis group 1 milk, Se^+/Le^+, containing all types of fucosyloligosaccharides, with α1$\rightarrow$2, α1$\rightarrow$3 and α1$\rightarrow$4 linkages.
2. Group 2 milk, Se^-/Le^+, without α1,2 fucosyloligosaccharides, as the result of lack of expression of the Se gene.
3. Group 3 milk, Se^+/Le^-, characterized by the absence of α1,4 fucosyloligosaccharides because of the inactivity of the Le gene.
4. Group 4 milk, Se^-/Le^-, containing only α1,3 fucosyloligosaccharides resulting from the expression of the Le-independent fucosyltranferase (Thurl *et al*., 1997; Coppa *et al*., 2011).

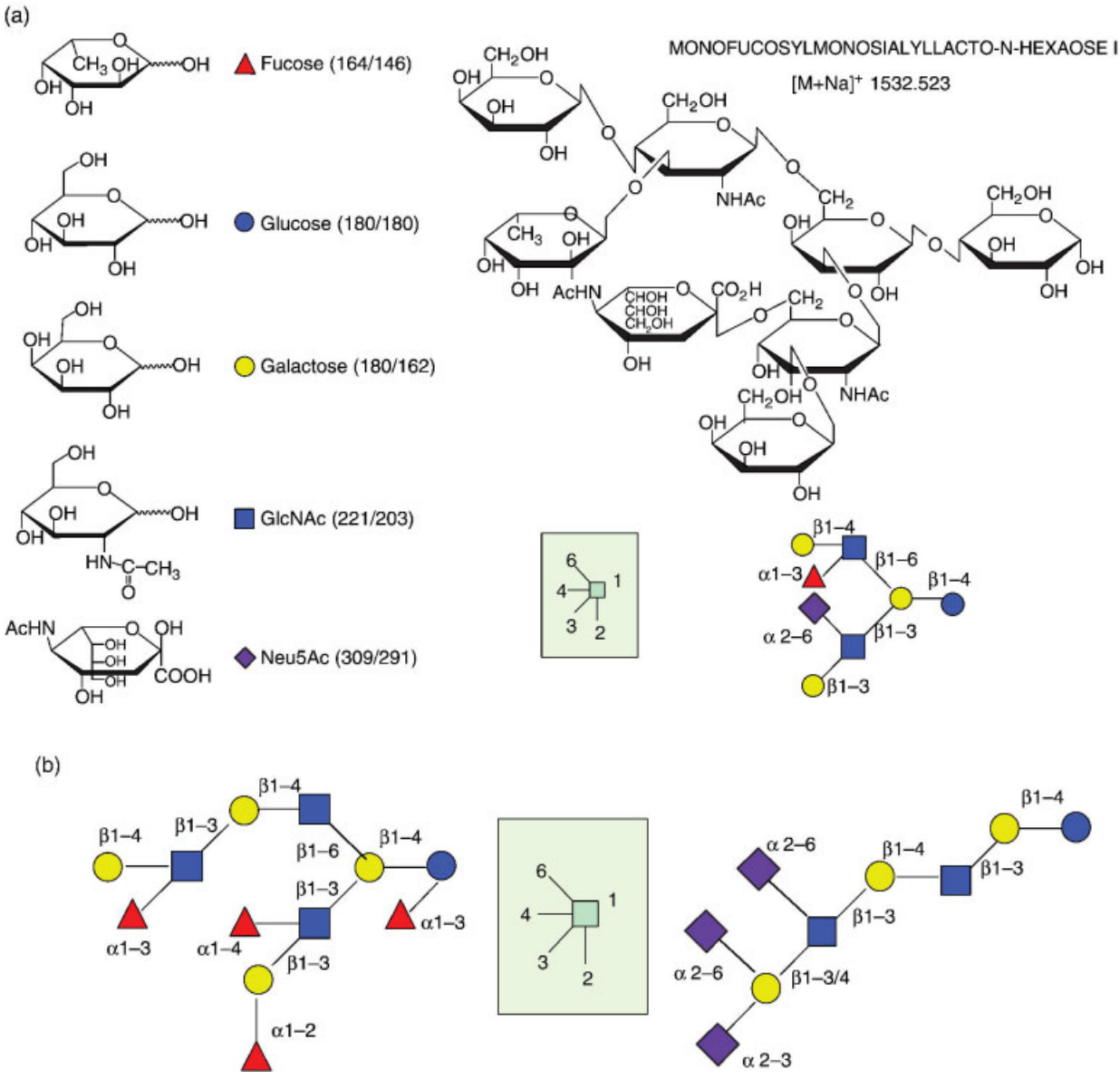


Figure 31.2. (a) Illustration of a human milk oligosaccharide (HMO) structure with key for interpreting symbols. (b) Examples of "branched" and "linear" HMO structures. Reproduced with permission from Wu *et al.* (2010). Copyright (2010) the American Chemical Society. For a color version of this figure, see Plate 31.1.

Although recognized in erythrocytes, Lewis glycan structures are also present in many secretions. The digestive track is probably a major, but not exclusive, site of Lewis motifs synthesis. After synthesis they are digested inside the digestive tract, reabsorbed, transported into the plasma, and only then adsorbed to the erythrocyte by a passive adsorption process (Rothenbacher *et al.*, 2004). Lewis groups are usually not detected on erythrocytes of newborns, but appear between 3 and 6 months and stabilize after 3 years. Interestingly the mother can administer large doses of Lewis structures to the infant during this period (Zivkovic *et al.*, 2011).

Because the human intestine does not express the luminal enzymes to cleave the α-glycosidic linkages of fucose and and sialic acid, as well as β-glycosidic linkages in the core HMO molecule, these molecules are resistant

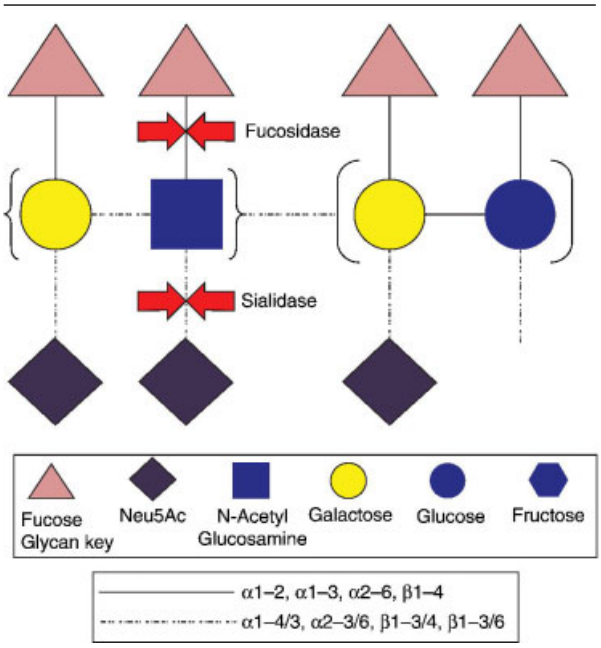


Figure 31.3. Basic structure of HMO molecules. Sites of fucosidase and sialidase activity are denoted by arrows. Reproduced from Sela (2011), with permission of Elsevier. For a color version of this figure, see Plate 31.2.

to enzymatic hydrolysis in the intestine (Engfer *et al*., 2000). Approximately 10% of the maternal caloric input for milk production would be expended synthesizing non-nutrient glycans, which seems counterintuitive (Newburg & Walker, 2007), but indigestible dietary glycans are known to enrich a beneficial microbiota containing bifidobacteria – the prebiotic effect. However, additional effects of HMOs other than just serving as prebiotics can be perceived since about 90% of all HMOs are found intact and not metabolized in the infant's feces (Bode, 2009). HMOs may possess antiadhesive effects that reduce the binding of pathogenic bacteria to colonocytes (Newburg, 2009). HMOs have modulating effects on immunologic processes at the level of gut-associated lymphoid tissue (Guarner, 2009) and may also decrease intestinal permeability in preterm infants in a dose-related manner in the first postnatal month (Taylor *et al*., 2009). Others have suggested that HMOs are an important source of *N*-acetylneuraminic acid, an essential monosaccharide during the period of neonatal brain development and myelination (Wang & Brand-Miller, 2003).

Bacterial surfaces have oligosaccharide-binding proteins, including lectins, responsible for adhesion to the host's epithelial surface. Some of these glycan-binding determinants are also part of HMOs, suggesting that various HMOs serve as soluble ligand analogs, blocking pathogen adhesion and protecting the breast-fed infant against infections and diarrhea (Newburg *et al*., 2005). Antiadhesive activity of free HMOs has been described for several microorganisms including pathogenic bacteria and viruses (Bode, 2009; Chichlowski *et al*., 2011). Another protecting mechanism has been observed recently. After treatment of Caco-2 cells with 3′-sialyllactose, one of the major oligosaccharides in human milk made a 90% reduction in the adhesion of enteropathogenic *Escherichia coli* to the epithelial surface of Caco-2. This was observed possibly due to the glycome-modifying effects by changing the expression of intestinal epithelial cell surface glycans (Angeloni *et al*., 2005).

Infants are born sterile but bacterial colonization of the gut occurs rapidly after birth. In breast-fed infants, bifidobacteria frequently predominate in the gastrointestinal tract with several beneficial effects to the newborn, including inhibition of the growth of pathogenic microorganisms and modulation of mucosal physiology, barrier function and systemic immunologic and inflammatory responses as recently stressed by the Committee on Nutrition of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (Agostoni *et al*., 2004). The "bifidogenic effect" of *N*-acetylglucosamine-containing oligosaccharides was identified over 50 years ago (Gauhe *et al*., 1954). However not all bifidobacteria isolates have the ability to grow vigorously on HMOs as a sole carbon source and different bifidobacterial species appear to have developed different strategies to consume specific HMOs (Zivkovic *et al*., 2011). A cluster of genes dedicated to the import and use of HMOs has been identified in *Bifidobacterium longum* subsp. *infantis*, a subspecies that commonly inhabits the infant gastrointestinal tract, allowing the introduction of lower-molecular-weight oligosaccharides into the cell. These oligosaccharides are then catabolized by a group of glycosidases before entry of the monosaccharides into central metabolic pathways (Sela *et al*., 2008). In contrast, other bacteria, including the closely related adult-type bifidobacteria *Bifidobacterium longum* subsp. *longum*, are deficient in this locus. Both *Bifidobacterium breve* and *B. longum* subsp. *longum* are able to consume free LNnT (lacto-*N*-neotetraose) from an HMO pool, whereas *B. breve* can also grow on the various monomer constituents of HMOs (Locascio *et al*., 2007; Ward *et al*., 2007). *Bifidobacterium bifidum* exports fucosidases and lacto-*N*-biosidase to remove lacto-*N*-biose from the HMO structure (Ward *et al*., 2007), internalize the free lacto-*N*-biose, and catabolize it intracellularly. These different strategies suggest a possible mechanism for niche partitioning

Table 31.6. Fatty acid composition of mature human milk.*

Fatty acid	Median	Range
6:0	0.28	0.03–0.69
8:0	0.66	0.11–1.76
10:0	3.00	0.57–6.15
12:0	9.78	2.14–34.90
14:0	8.84	1.57–27.61
16:0	21.90	12.68–29.21
18:0	6.35	1.08–9.68
20:0	0.20	0.03–0.91
22:0	0.09	0.00–0.34
24:0	0.07	0.00–0.31
MCSFA	22.41	4.65–67.92
LCSFA	38.35	25.53–47.15
18:3 *n*-3	0.92	0.27–2.71
20:5 *n*-3	0.05	0.00–1.18
22:5 *n*-3	0.12	0.00–0.31
22:6 *n*-3	0.21	0.08–1.63
LCPUFA *n*-3	0.38	0.10–1.68
n-3	1.30	0.16–3.08
14:1 *n*-5	0.28	0.03–0.69
18:2 *n*-6	12.67	3.51–30.03
18:3 *n*-6	0.09	0.00–0.33
20:2 *n*-6	0.31	0.08–0.99
20:3 *n*-6	0.36	0.18–0.78
20:4 *n*-6	0.42	0.19–0.99
22:4 *n*-6	0.09	0.00–0.50
22:5 *n*-6	0.04	0.00–0.18
LCPUFA *n*-6	1.23	0.59–3.25
n-6	14.08	4.14–31.35
16:1 *n*-6	2.33	0.65–5.89
18:1 *n*-7	2.89	0.50–7.63
n-7	5.36	1.65–10.34
18:1 *n*-9	25.20	7.17–40.05
20:1 *n*-9	0.35	0.06–1.10
20:3 *n*-9	0.05	0.00–0.20
24:1 *n*-9	0.05	0.00–0.46
n-9	25.60	7.28–40.45
MUFA	31.48	9.54–46.60
PUFA	15.49	5.03–32.34
LCPUFA	1.64	1.02–3.96
LC *n*-6/LC *n*-3	3.18	0.40–9.85
n-6/*n*-3	10.36	3.54–101.03
20:3 *n*-9/20:4 *n*-6	0.12	0.00–0.47
20:4 *n*-6/22:6 *n*-3	1.97	0.15–5.71
18:2 *n*-6/18:3 *n*-3	13.44	4.77–42.89

*Values are expressed in mol% for pooled data ($N=55$) for mature milk from women in The Netherlands, Caribbean region, Jerusalem and Tanzania.

among the different bifidobacterial species within the developing infant gastrointestinal tract microbiota (Zivkovic *et al*., 2011).

Additionally, several species of lactic acid bacteria from the mother's intestine are thought to travel to her mammary glands inside white blood cells (Petherick, 2010) and breast milk can be a natural source of beneficial lactic acid bacteria for the newborn gut (Martin *et al*., 2003). Most of these species inhibit pathogenic bacteria by secreting hydrogen peroxide and compounds called bacteriocins (Petherick, 2010).

31.4.4 Milk fat

31.4.4.1 Milk fat composition

Fat represents about 50% of the energy content of mature human milk. It is the second most prevalent class of nutrients in human milk, after lactose, averaging 15–20 g/L in colostrum and 38–40 g/L in mature milk. A wide range of fat contents was found in an analysis of 2554 samples from 224 Danish mothers taken over 33 months of lactation (18.4 g/L at percentile 2.5 up to 89.0 g/L at percentile 97.5), while the relative variation was smaller for protein and much less for lactose (Michaelsen *et al*., 1990).

Fat is important in the provision of energy as well as being the nutrient vehicle for fat-soluble vitamins and essential fatty acids. Although the amount of fat in human milk is not very different from that in cow milk, the types of fatty acids are very different. Human milk fat is high in unsaturated fatty acids and polyunsaturated fatty acids (PUFAs), particularly the essential fatty acids linoleic (18:2 *n*-6) and α-linolenic (18:3 *n*-3) (Table 31.6). Arachidonic acid (20:4 *n*-6) is supplied in sufficient amounts to support the structure and function of neural and brain tissue in fetal life and during postnatal development. Long-chain PUFAs have been claimed to be conditionally indispensable for the neonate, especially for the preterm baby. Preterm infants may have difficulty synthesizing long-chain PUFAs from their precursors in sufficient amounts. Linoleic and linolenic acid, both essential fatty acids, are present in preterm infant milk formula at around 0.5 g/dL and 0.1 g/dL, respectively.

Human milk fat is more efficiently absorbed than cow milk-based formulae partly due to the presence of bile salt-stimulated human milk lipase. However, this is probably not of great significance except in premature

LC, long-chain; LCPUFA, long-chain polyunsaturated fatty acids; LCSFA, long-chain saturated fatty acids; PUFA, polyunsaturated fatty acids; MCSFA, medium-chain saturated fatty acids; MUFA, monounsaturated fatty acids.

Source: reproduced from German & Dillard (2006), with permission of Taylor & Francis.

infants, whose gastrointestinal enzymic function is poorly developed. Most recently, cow milk infant formulae (but not goat milk formulae) contain fats in the form of vegetable oils, which are very different in their fatty acid structure from those in cow milk or human milk. Human milk has a higher level of cholesterol than cow and goat milk, where the corresponding cholesterol concentrations of the three species are 16, 14, and 10 mg/100 g milk. Relatively high serum concentrations of cholesterol are a feature of many mammals during the suckling period. The serum cholesterol concentration of an infant fed human milk is higher than that of one fed cow milk formulae, where the fat has been replaced with vegetable oils (Morgan, 2006).

31.4.4.2 Fatty acids of human milk in the health and cognitive development of children

Human milk supplies the suckling infant with a complex array of lipids that contribute to 50% of the energy intake with mature human milk. Most fatty acids (98–99%) are esterified as triacylglycerols and only a minimal fraction is complexed into phospholipids, monoacylglycerols, and diacylglycerols. Cholesterol (0.1–0.2 g/L) is also included in the lipid extract (Agostoni *et al*., 1999). Besides its lipidic energetic content, human milk provides all the dietary essential fatty acids, i.e., linoleic acid (LA; 18:2 *n*-6) and α-linolenic acid (ALA; 18:3 *n*-3), as well as their long-chain polyunsaturated fatty acids (LCPUFAs), including arachidonic acid (AA; 20:4 *n*-6) and docosahexaenoic acid (DHA; 22:6 *n*-3) to support the growth and development of the breast-fed infant (Table 31.6) (Innis, 2007).

The two 18-carbon essential fatty acids, LA (parent of the *n*-6 series of fatty acids) and ALA (parent of the *n*-3 series), rely for much of their biological activity on being converted to 20- and 22-carbon fatty acids, LCPUFAs, through a series of desaturases and elongases. In the case of the *n*-6 series, this primarily refers to the conversion of LA to the 20-carbon AA (20:4 *n*-6); for the *n*-3 series, ALA must be converted to a 20-carbon fatty acid, eicosapentaenoic acid (EPA; 20:5 *n*-3), and a 22-carbon fatty acid, DHA (22:6 *n*-3). When diets contain only 18-carbon essential fatty acids (LA, ALA), levels of the *n*-3 and *n*-6 LCPUFAs tend to be regulated by the relative amounts of these fatty acids in the diet. However, providing increasing amounts of ALA is not an effective strategy for increasing tissue DHA content. Regarding the increase in tissue DHA content, available data suggest that diets low in ALA are more functional as long as the level of LA in the diet is also low (Gibson *et al*., 2011).

It is known that the levels of unsaturated fatty acids in human milk are highly dependent on the lactating woman's diet, particularly LA, ALA and DHA, but also from her long-term intake since milk LCPUFAs are largely derived from endogenous body stores (Koletzko *et al*., 2001; Innis, 2007). Therefore, the mother's status and a diet low in *n*-6 PUFAs allow better endogenous conversion of ALA to *n*-3 LCPUFAs and permit better accumulation of *n*-3 LCPUFAs into tissues, including milk (Gibson *et al*., 2011).

Changes over decades in the intake of *n*-6 and *n*-3 PUFAs, with a significant increase in the ratio of LA to ALA, are being observed. The mean LA content of breast milk in the USA increased from 6 to 15% of total fatty acids between 1944 and 1990, and has since remained at approximately 16%, while the ALA content has declined (Fig. 31.4) (Ailhaud *et al*., 2006).

A similar pattern has also been reported in the breast milk of Australian women between 1981 and 2000 (Gibson & Kneebone, 1981; Makrides *et al*., 2000). In 2002, Smit *et al*. reported a large biological variation in the composition of 28 fatty acids in 465 mature human milk samples from five regions of the world, with the *n*-6/*n*-3 ratio varying impressively between 3.5 and 101. This variability and changes in human milk fatty acids result in considerable differences in the intake and blood levels of *n*-6 and *n*-3 fatty acids among breast-fed infants (Innis, 2007).

AA, EPA, and DHA play central roles in infant growth, neural development, and immune function; the supply of LCPUFAs has been related to functional outcomes of the infant such as visual acuity and development of cognitive functions. LCPUFAs are processed to powerful promoters of eicosanoids such as prostaglandins and leukotrienes, signal molecules and regulators of many biological systems (Simopoulos, 2010). One of the key features of *n*-6 fats is that they give rise to proinflammatory eicosanoids, which have been implicated in the development of allergies and asthma (Gibson *et al*., 2011). In addition, *n*-6 fats promote the differentiation of preadipocytes, and exposure to excessive *n*-6 fats during the period of fat cell formation (*in utero* and in early infancy) has the potential to promote excess fat deposition in early life (Ailhaud *et al*., 1992). This observation was corroborated by several studies that implicated the increasing dominance of *n*-6 to *n*-3 fats in Western diets as a contributing factor to the increased incidence of childhood obesity (Ailhaud & Guesnet, 2004; Ailhaud *et al*., 2006, 2007; Massiera *et al*., 2010). Hence, consideration needs to be given to replacing the *n*-6 fatty acids of vegetable oils and spreads with monounsaturated fatty acids in order to achieve blends with around 5% of total fatty acids as PUFAs and with an LA/ALA ratio of around 2 : 1 or less (Gibson *et al*., 2011).

Interestingly, there have been many indications for considerable interindividual variation in the capacity for endogenous formation of LCPUFAs (Simopoulos, 2010). The key enzymes in *n*-6 and *n*-3 fatty acid metabolism are the Δ^5 and Δ^6 desaturases, which are encoded by the *FADS1* and *FADS2* genes, respectively, located on the desaturase gene cluster.

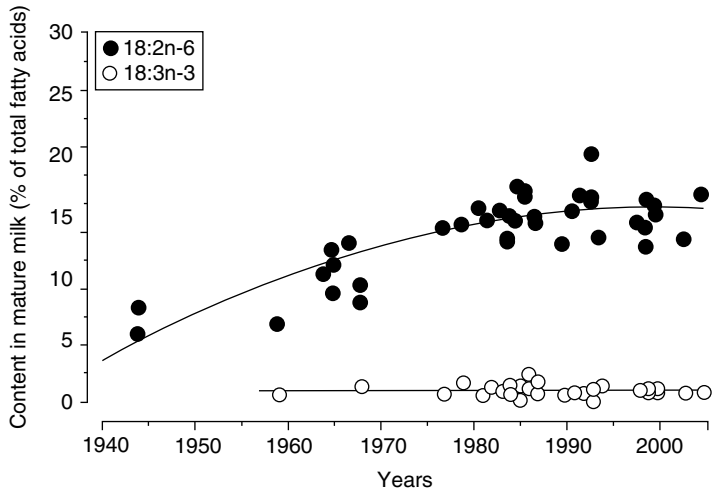


Figure 31.4. Linoleic acid (●) and α-linolenic acid (○) content in mature breast milk of US women from 1944 to 2005. For linoleic acid (18:2 *n*-6), the best regression was a two-order polynomial fit $y=-0.0029x^2+11.9x-1189$, $N=40$, $r=0.82$, $P<0.001$. For α-linolenic acid (18:3 *n*-3), no significant relationship was noted ($y=-0.00035x^2+1.39x-1373$, $N=28$, $r=0.14$, NS). Reproduced from Ailhaud *et al.* (2006), with permission of Elsevier.

The fatty acid composition of serum phospholipids is reportedly genetically controlled by the *FADS1* and *FADS2* gene cluster. Subjects carrying the minor alleles of the single nucleotide polymorphisms (SNPs) rs 174544, rs 174553, rs 174566, rs 174561, rs 174568, rs 968567, rs 99780, rs 174570, rs 2072114, rs 174583, and rs 174589 were associated with increased levels of 18:2 *n*-6, 20:2 *n*-6, 20:3 *n*-6, and 18:3 *n*-3, and decreased levels of 20:4 *n*-6, 22:4 *n*-6, 20:5 *n*-3, and 22:5 *n*-3 (Schaeffer *et al*., 2006). These observed genetic variants indicate a difference in the conversion ability of *n*-6 and *n*-3 fatty acids catalyzed by the Δ^5 and Δ^6 desaturases, which suggests that individuals may require different amounts of dietary PUFAs or LCPUFAs to achieve comparable biological effects (Simopoulos, 2010).

Essential fatty acids of breast milk have also been shown to be influenced by the mother's genetic variants of *FADS1* and *FADS2*, with significantly lower 14:0, AA and EPA, but higher 20:2 *n*-6 in the minor allele homozygotes of rs 174553 (GG), rs 99780 (TT), and rs 174583 (TT) and lower AA, EPA, 22:5 *n*-3, and DHA in the minor allele homozygotes GG of rs 174575. These results indicate a robust association between minor alleles of the four SNPs and lower AA and other *n*-6 fatty acids relative to precursor LA. Similar results have been found for the *n*-3 series (Xie & Innis, 2008).

Substantial amounts of DHA and AA accumulate in the human brain during the first postnatal months, and infants who are breast-fed have higher concentrations of DHA and AA than infants fed unsupplemented formulae. Children with a longer duration of breastfeeding have significantly higher mental scores than children with low breastfeeding duration exposed to low levels. The effect of breastfeeding on child neurodevelopment seems to be driven in part by the LCPUFA content of breast milk. Higher levels of various *n*-3 PUFAs, including DHA, and high ratios between *n*-3 and *n*-6 PUFAs (ALA/LA and DHA/AA) have been positively associated with infant mental development, especially among children with high levels of cumulative breastfeeding (Guxens *et al*., 2011). Additionally, this effect seems to be moderated by *FADS2* polymorphisms in children (Caspi *et al*., 2007; Steer *et al*., 2010).

The PUFA composition of breast milk may also have a beneficial effect on asthma and atopy. The association between exclusive breastfeeding and asthma may also be modified by the genetic variants of *FADS* genotypes in children (Standl *et al*., 2012). The same study suggests that only minor allele carriers benefit from exclusive breastfeeding in regard to asthma development, while homozygous major allele carriers have no advantage in this respect (Standl *et al*., 2012).

31.4.5 Milk micronutrients

31.4.5.1 Iron and minerals

Human milk contains only small amounts of iron (Table 31.5), but this has little nutritional consequence in healthy term infants during the initial months of life. A normal healthy fetus stores iron in its liver in the last trimester of pregnancy (Morgan, 2006), whereby a full-term

infant can maintain satisfactory hemoglobin levels without any other sources of iron for about the first 6 months of life. Although breast milk is noticeably low in iron (76 μg/dL), an extra dietary source is unnecessary until the stores are exhausted at around 6 months. This is because the iron in breast milk is well absorbed (50–70%, compared with 10–30% from cow milk) and an infant has iron stores (Morgan, 2006).

The iron in human milk is mostly present in the fat globule membranes of the lipid fraction and the remainder (30%) is found in lactoferrin. The iron content of human milk is not affected by maternal stores. Lactoferrin is an iron-binding protein that has a role in facilitating the absorption of iron and other nutrients; it competes with bacteria for nutrients and thus inhibits pathogen multiplication in the infant gut. On the other hand, supplementation with exogenous or dietary iron sources is needed from around 6 months after birth. Infant milk formulae and many infant food products contain added iron, which must ensure an appropriate dietary intake for the infants. Other foods, such as properly prepared red meat, may be included in baby foods to provide additional sources of bioavailable iron.

Other essential minerals in human milk, such as calcium, phosphorus, magnesium, sodium and zinc, are needed for growth and appropriate body metabolism of infants. Calcium, magnesium and phosphorus are highly important for bone growth. Zinc is essential in body metabolism, enzymes, and wound healing, and is also present in plasma, erythrocytes, leukocytes, and platelets (Underwood, 1977). The concentrations of sodium, calcium and zinc in mature human milk are 15, 35 and 0.3 mg/100 g, respectively (Table 31.5).

31.4.5.2 Vitamins

31.4.5.2.1 Vitamins A, C, K and others in human milk

The essential vitamins in human milk are efficiently absorbed and utilized for healthy term infants up to the age of around 4–6 months. Levels of water-soluble vitamins are likely to be influenced by the mother's diet and her own vitamin status, while those of fat-soluble vitamins may be less influenced by the mother's status. If human milk is still the main food, the adequacy of the provision of certain vitamins to an infant after 6 months of age may not be secure. Guidelines on the provision of supplements have been devised for the UK by the Department of Health (1994):

> *Breast fed infants under six months do not need vitamin supplementation provided the mother has an adequate vitamin status during pregnancy. From the age of six months infants receiving breast milk as their main drink should be given supplements of vitamins A and D.*

A Department of Health vitamin supplement of five drops daily contains 7 μg of vitamin D, 200 μg of vitamin A and 20 mg of vitamin C (Morgan, 2006).

The vitamin K content of human milk varies widely, between 1 and 10 μg/L. Since newborn infants have no colonic flora synthesizing vitamin K, the infant's ability to absorb vitamin K is variable and plasma levels are low (Morgan, 2006). Approximately 2% of newborn infants show evidence of a hemorrhagic bleeding disease in the intestine due to inadequate vitamin K-dependent coagulation factors. Oral vitamin K (1 mg) is now recommended for all healthy newborn infants in the UK, with a further four oral doses being offered at 2-weekly intervals to human milk-fed infants. Vitamin K content of cow milk is higher than that of human milk, and a typical whey type formula contains 7 μg/dL, which is close to the upper limit in human milk. However, hemorrhagic disease due to inadequate vitamin K almost never occurs in cow milk formula-fed infants. Additional details of concentrations of other selected vitamins in human milk including folate, thiamine, riboflavin and vitamin B_{12} are provided in Table 31.5.

31.4.5.2.2 Vitamin D in human milk

Vitamin D (calciferol) content in human breast milk is very low (0.01 μg) in mature human milk (Table 31.5), indicating that a dietary source of this substance is teleologically unimportant. Infants have a store of vitamin D at birth and additional vitamin D may be supplied from the action of sunlight (ultraviolet or UV light) on 7-dehydrocholesterol in the skin, so infants should spend time exposed to sunlight. The amount of exposure to UV light will differ between infants and the location or country in which the infant was born.

The active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 (cholecalciferol) or $1,25(OH)_2D$, increases intestinal calcium absorption and facilitates mineralization of the skeleton. Without $1,25(OH)_2D$, the body cannot absorb calcium and phosphorus adequately; in response, blood parathyroid hormone increases (secondary hyperparathyroidism), the skeleton loses mineral content (secondary osteoporosis), and new bone is not adequately mineralized (rickets or osteomalacia) (Kovacs, 2008). Moreover, $1,25(OH)_2D$ acts in a wide range of tissues, including the liver, pancreas, brain, lung, breast, skin, muscle, and adipose tissue. Because of the wide-ranging involvement of vitamin D in many systems of the body, an inadequate vitamin D supply may have other potential health impacts in early and later postnatal life in addition to rickets and osteomalacia, but is still not well understood (Kovacs, 2008; Lucas *et al*., 2008; Prentice *et al*., 2008).

Vitamin D deficiency in adults, characterized by a low plasma 25-hydroxyvitamin D (25OHD) level, is associated

Table 31.7. Definition of 25OHD status.

25OHD level	CPS* (ng/mL)	ES† (ng/mL)
Deficient	<10	<20
Insufficient	20–30	21–29
Optimal‡	30–90	>30
Pharmacological (potential adverse effects)	>90	
Potentially toxic	>200	

Note: 1 ng/mL = 2.5 nmol/L.

*According to the Canadian Paediatric Society (CPS, 2007).

†According to the Endocrine Society (Holick *et al*., 2011).

‡Recently, the Institute of Medicine (2010) concluded that people should aim for blood levels of 20 nmol/L, but this "aimed" value has been criticized by several vitamin-D proponents as too low (Maxmen, 2011).

with decreased calcium absorption from the gut and a tendency toward hypocalcemia and can be defined according to Table 31.7. There are few existing data on which to establish the normal range of 25OHD in infancy; however, previous studies point out that the optimal range is similar to that of adults (CPS, 2007).

Globally, vitamin D-deficient rickets in infants and children remains prevalent, ranging from 9 to 70% in Asia, the Middle East, and Africa, and is reemerging in developed countries in ethnically diverse, dark-skinned and prolonged breast-fed individuals without vitamin D supplementation (Prentice, 2008). In 2008, the American Academy of Pediatrics, highlighting the key role that vitamin D plays in infant health, introduced new guidelines for oral infant supplementation with vitamin D_3, increasing their recommendations from 200 to 400 IU/day (from 5 to 10 μg/day) for breastfeeding infants and infants receiving less than 1 L of formula daily (Wagner & Greer, 2008). The Canadian Paediatric Society recommends that total vitamin D intake during the first year should be 400 IU/day in full-term infants, with an increase to 800 IU/day from all sources between October and April for areas north ofthe 55th parallel and for areas between the 40th and 55th parallel in individuals with risk factors for vitamin D deficiency other than latitude alone. In addition, they recommend considering vitamin D 2000 IU/day for pregnant and lactating women, especially in winter (CPS, 2007). In fact a vitamin D intake of approximately 400 IU/day has been shown to maintain serum 25OHD concentrations above 50 nmol/L in breast-fed infants (Wagner *et al*., 2006).

Multiple issues concern the physiology of vitamin D in lactation, including the vitamin D content in the milk and its relationship to maternal vitamin D status, and the resulting nutritional status of the infant (Brannon & Picciano, 2011). Vitamin D passes readily from the mother's serum into breast milk, 25OHD passes very poorly, and 1,25$(OH)_2$D does not appear to pass at all (Kovacs & Kronenberg, 1997). Different sampling and analytical approaches to the measurement of vitamin D and its metabolites in human milk have been reported in five studies (Brannon & Picciano, 2011). Vitamin D content in human milk ranges from 39 pg/mL (Hollis *et al*., 1981) to 579 pg/mL (Specker *et al*., 1985) and is relatively low (~18%) compared with maternal serum vitamin D levels (Hollis *et al*., 1986). 25OHD content ranges from 84 pg/mL (Kamao *et al*., 2007) to 1900 pg/mL (Olafsdottir *et al*., 2001). Human milk 25OHD level is only 0.9% compared with maternal serum 25OHD, a lower ratio when compared with the ratio between vitamin D levels in human milk and maternal serum. Human milk 1,25$(OH)_2$D levels range from 5.1 pg/mL (Hollis *et al*., 1981) to 22 pg/mL (Takeuchi *et al*., 1989). A recent study showed that vitamin D and $25OHD_3$ content does not vary from colostrum to transitional to mature milk, whereas $25OHD_2$ decreases 50% from colostrum to transitional milk and remains low in mature milk (Kamao *et al*., 2007). Vitamin D-binding protein is also present in human milk at 3.3%, the level found in maternal serum and is unrelated to the vitamin D or 25OHD content (Hollis *et al*., 1986); however its origin is not known. Thus, total vitamin D activity in "normal" human milk is relatively low: the average across studies is 544 pg/mL (~22 IU/L), which would provide approximately 16 IU/day to the exclusively breast-fed infant in the first 6 months, assuming an average daily amount of milk produced of 750 mL.

In lactating mothers taking 400 IU/day vitamin D supplementation, it was found that human milk contains 33–68 IU/L antirachitic activity (Hollis *et al*., 1986). In another study, with the same dosage, the average breast milk vitamin D content was around 38 IU/L (Hollis & Wagner, 2004), which is far below the recommended daily vitamin D level for infants of 400–800 IU (CPS, 2007; Wagner & Greer, 2008).

Lactating women given high oral doses of vitamin D or exposed to UV light can produce milk that contains high levels of antirachitic activity. This increase in breast milk vitamin D activity is almost totally due to the parent compound, vitamin D, which passes efficiently to the milk, and not the major circulating form, 25OHD (Hollis & Wagner, 2011). The transfer of vitamin D into human milk allowed antirachitic activity as high as 7600 IU/L in a mother maintained on 100 000 IU/day vitamin D_2 (ergocalciferol) for

treatment of hypoparathyroidism (Greer *et al*., 1984a). Maternal supplement with vitamin D_2 or vitamin D_3 (1000 IU/day) doubled the 25OHD breast milk content with little effect on vitamin D. In the same study, with a higher supplemental dose of vitamin D_3 (2000 IU/day), breast milk 25OHD increased around 2.3 times and again the effect on milk vitamin D was very small. The effect of vitamin D (D_2 or D_3) supplements was higher when the baseline milk 25OHD and vitamin D content was smaller (Ala-Houhala *et al*., 1988).

By 48 hours after total body UV-B exposure (equivalent to 30 min of sunshine at noon on a clear summer day in fair-skinned women), an increase in vitamin D_3 concentrations in serum (from 1.0 to 22.9 ng/mL, i.e., 40 to 920 IU/L) and milk (from 0.15 to 1.78 ng/mL, i.e., 6 to 71 IU/L) was observed in lactating white women, followed by a rapid decline in both fluids due to the relatively short half-life of vitamin D_3. In these same subjects, circulating $25OHD_3$ concentrations also increased from 13.9 to 20.5 ng/mL and remained significantly elevated for at least 14 days. There was no significant change, however, in milk $25OHD_3$ concentrations during this period (Greer *et al*., 1984b).

31.5 INFANT FORMULAE AND ALTERNATIVE FEEDING

The recommended dietary intake for infants in the first 6 months of life is based on nutrient analysis of human breast milk and average volumes of milk consumption by healthy, full-term, exclusively breast-fed infant (Yates, 2006). Thus, the composition of human milk is also the model for the preparation of infant formula. Usually the composition of infant formulae does not take into account the complexity and the gradually changing pattern of human milk composition during the breastfeeding period. These are the main reasons for the differences found in the growth pattern and health of breast-fed infants when compared with formula-fed infants (Lönnerdal, 2008). In addition to nutrients, human milk also contains hormones, growth factors, immunoglobulins, cytokines, enzymes, sphingomyelin, and specific oligosaccharides that support both the growth and the passive defenses of the infant and which are lacking in infant formulae (Le Huerou-Luron *et al*., 2010).

Regarding the absence of HMOs in bovine milk-based infant formulae, several infant formula-producing companies have searched for inexpensive alternatives and developed mixtures of galactooligosaccharides (GOS) and fructooligosaccharides (FOS) or inulin that mimic the prebiotic effects of human milk and promote a bacterial microflora that closely resembles that of breast-fed infants. However, GOS and FOS or inulin are structurally much simpler than the oligosaccharides occurring naturally in human milk (HMOs). Considering that most of the postulated effects of HMOs are highly structure-specific, infant formula oligosaccharides may have different effects than HMOs. Extensive research is needed to clarify the specific effects of these "artificial" glycans and, more so, to understand the mechanisms by which HMOs benefit the breast-fed infant. To provide formula-fed infants with the same benefits that breast-fed infants receive with their mother's milk, there is an urgent demand for alternative HMO-like glycans to supplement infant formulae (Bode, 2009).

Although it is not possible to mimic the exact nutritional and non-nutritional components of human milk, commercially prepared cow milk-based infant term formulae were becoming increasingly popular in the 1940s. In the manufacture of the majority of first age infant formulae, bovine milk is used as the base ingredient, but important changes in the composition of infant milk formulae have occurred in the last decade. The average energy and nutrient contents of a typical infant milk formula based on cow milk are displayed in Table 31.5. Since the 1950s, butterfat from cow milk has been generally replaced with a suitable blend of oils from a vegetable source, so that now all infant formulae contain blends based on vegetable oils. This has led to an increased intake of PUFAs in the formula-fed infant (Morgan, 2006).

Although bovine and human milks have similar calorie levels (~65–70 kcal/dL) (Fomon, 1993), their macronutrient profiles are quite different. Over the decades, the level of protein in cow milk-based formula has also been decreased, with reductions in electrolytes (especially sodium) and phosphate. This is partly in response to concerns over the potential renal solute load of feeds containing high protein and high solute. In addition to the alteration in protein content, the casein to whey ratio has been decreased from 82 : 18 to 40 : 60 (Morgan, 2006). The carbohydrate content is also adjusted by the addition of lactose or maltodextrins to maintain energy levels of modified formulae. Cow milk-based follow-on formulae can be fed to infants over 6 months and will constitute the main milk drink in this case. Follow-on formulae can be considered a complementary food and can enhance the mixed diet of an infant, where solid foods provided may be low in protein, energy or micronutrients compared with the nutrient profile of infant formula or human milk. Follow-on formula is higher in protein, some micronutrients and iron than term formula (Morgan, 2006).

Very low birthweight and preterm infants require special formulae in order to meet the increased energy and nutrient needs for low-birthweight infants. Low-birthweight formulae are based on guidelines recommended by several expert groups, such as the Association of the Food Industries for

Particular Nutritional Uses of the European Union (IDACE), the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the Life Sciences Research Office (LSRO) of the American Society for Nutritional Sciences (ASNS), the American Academy of Pediatrics Committee on Nutrition (AAPCON), and Tsang *et al*. (2005). In the UK, there are two types of formulae on the market. First, there are low-birthweight formulae designed for the nutritional management of infants in special care baby units. After the infant is discharged from the hospital, she or he may still be growing rapidly. The post-discharge infant formulae based on cow milk are available for the preterm infant who may not be receiving human milk. Infants having a family history of atopy (allergy) and/or already diagnosed with atopic disorders may be fed hypoallergenic formulae such as either soy protein based or partially hydrolysed modified cow milk protein. The chance of developing an allergy to soy protein may be similar to that of developing an allergy to cow milk protein. Dietetic advice is desirable for parents embarking on modification of the infant's milk diet. Modified or unmodified milk from sheep or goats or other mammals fed to human infants can remedy cow milk allergy in some cases, although more clinical trials beyond anecdotal reports are needed for clear verification in this regard. Readers are encouraged to read Chapter 21 for further information on infant formulae and their feeding, and Chapter 6 about milk protein allergy.

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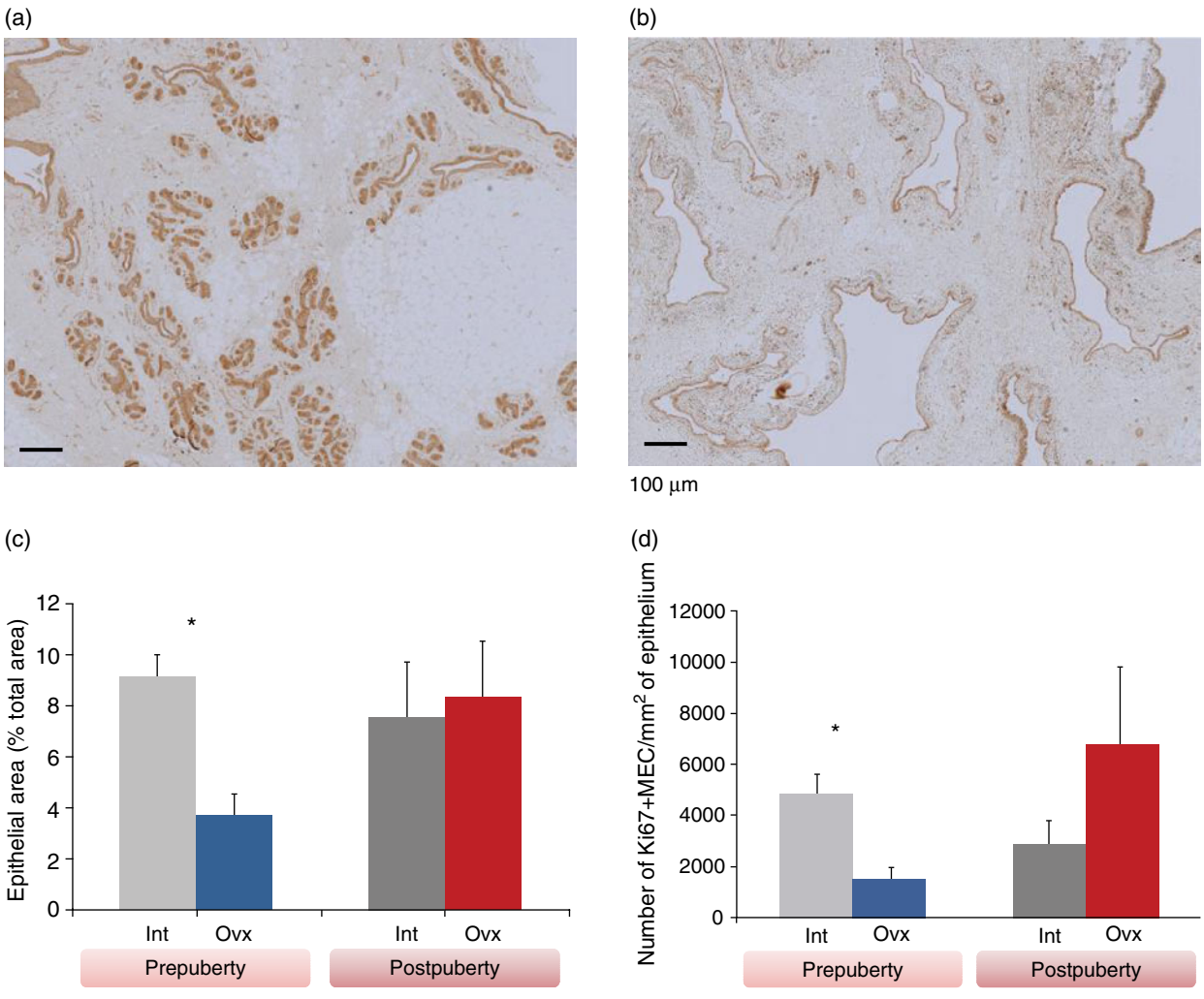


Plate 2.1. Effects of ovarian secretions on mammary gland development in young goats at puberty. Histological section of mammary gland obtained at 9 months of age from control intact young goats (a) and from young goats ovariectomized at 1 month of age (b) (bar = 100 μm). Ovariectomy before puberty affects mammary epithelium development (c) and proliferation of mammary epithelial cells (MEC) (d), but has no effect on these two parameters after puberty. * $P<0.05$. Int, intact control young goats; Ovx, ovariectomized young goats.

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Plate 3.1. Simple platform, side-by-side, milking parlor for goats at the AGROCAMPUS OUEST/INRA laboratory.



Plate 3.2. Buffalo milking with autonomous bucket machine milking.

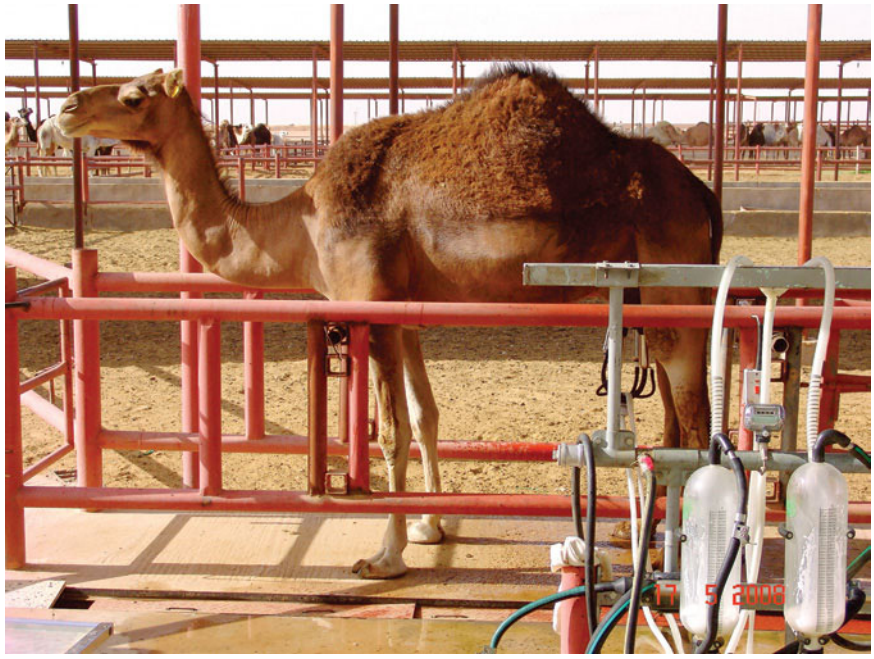


Plate 3.3. Camel milking stall and equipment in Saudi Arabia. Courtesy of Dr B. Faye.



Plate 3.4. Tandem milking parlor.

(a)



(b)



Plate 3.5. (a) Side-by-side milking parlor with rapid exit; (b) view of a cow in a side-by-side milking parlor. Access to the mammary gland is limited and observation of the cow reduced. Courtesy of Dairymaster.



Plate 3.6. Rotary parlor milking system.

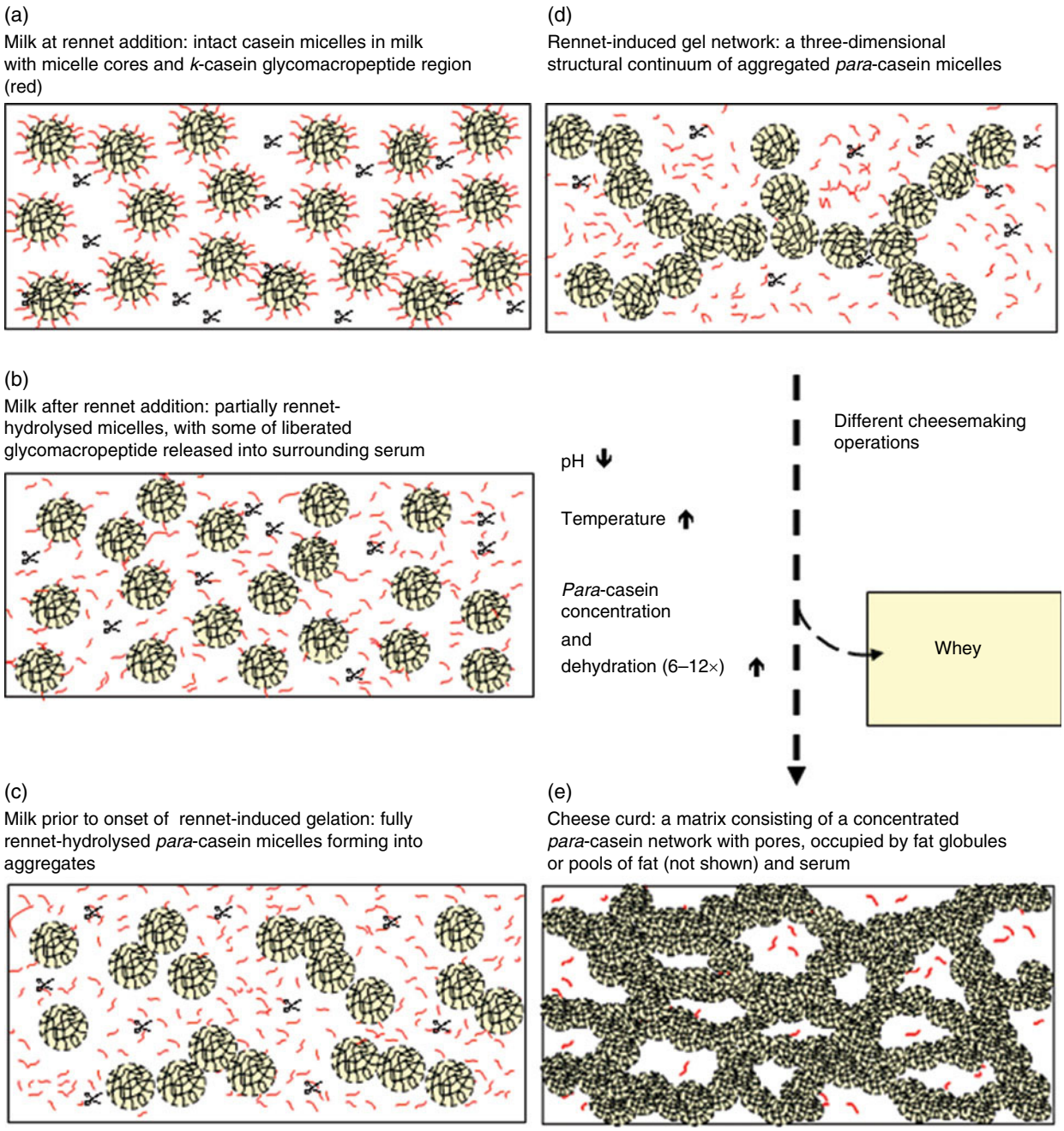


Plate 17.1. Schematic representation of the various stages involved in the formation of cheese curd from milk, starting from the initial mixture of casein micelles and added enzyme (rennet ✂) in the milk (a), and proceeding through rennet-induced hydrolysis of κ-casein (b, c), aggregation of *para*-casein micelles (c) and formation of *para*-casein gel network (d), which is dehydrated and concentrated into cheese curd (e).

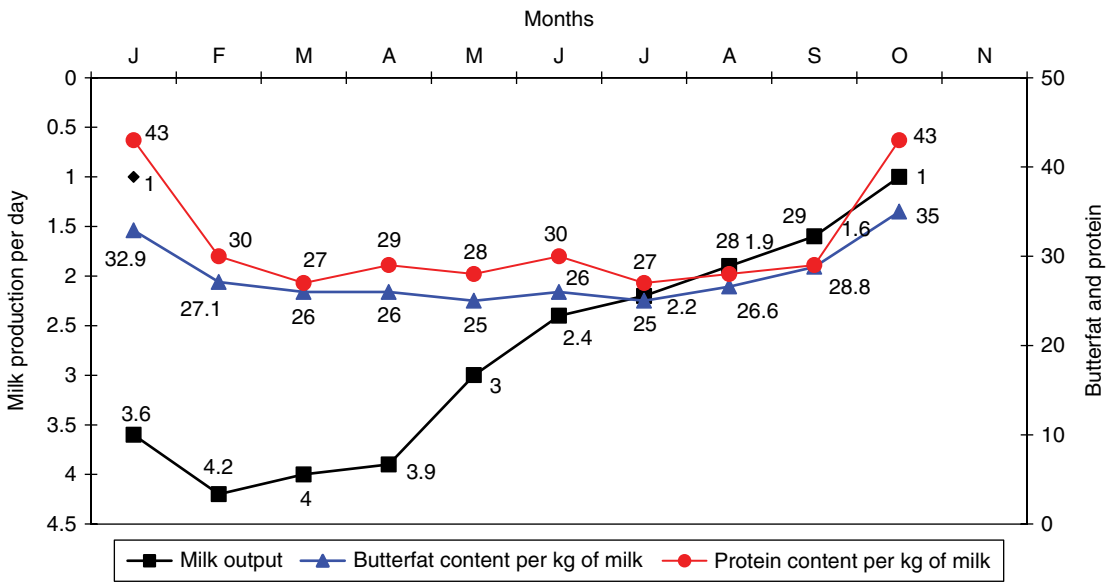


Plate 23.1. Changes in milk yield (kg) and butterfat and protein content (g/kg) during different stages of lactation in goats. Modified from Le Jaouen (1987), with permission of New England Cheesemaking Supply Company (www.cheesemaking.com).

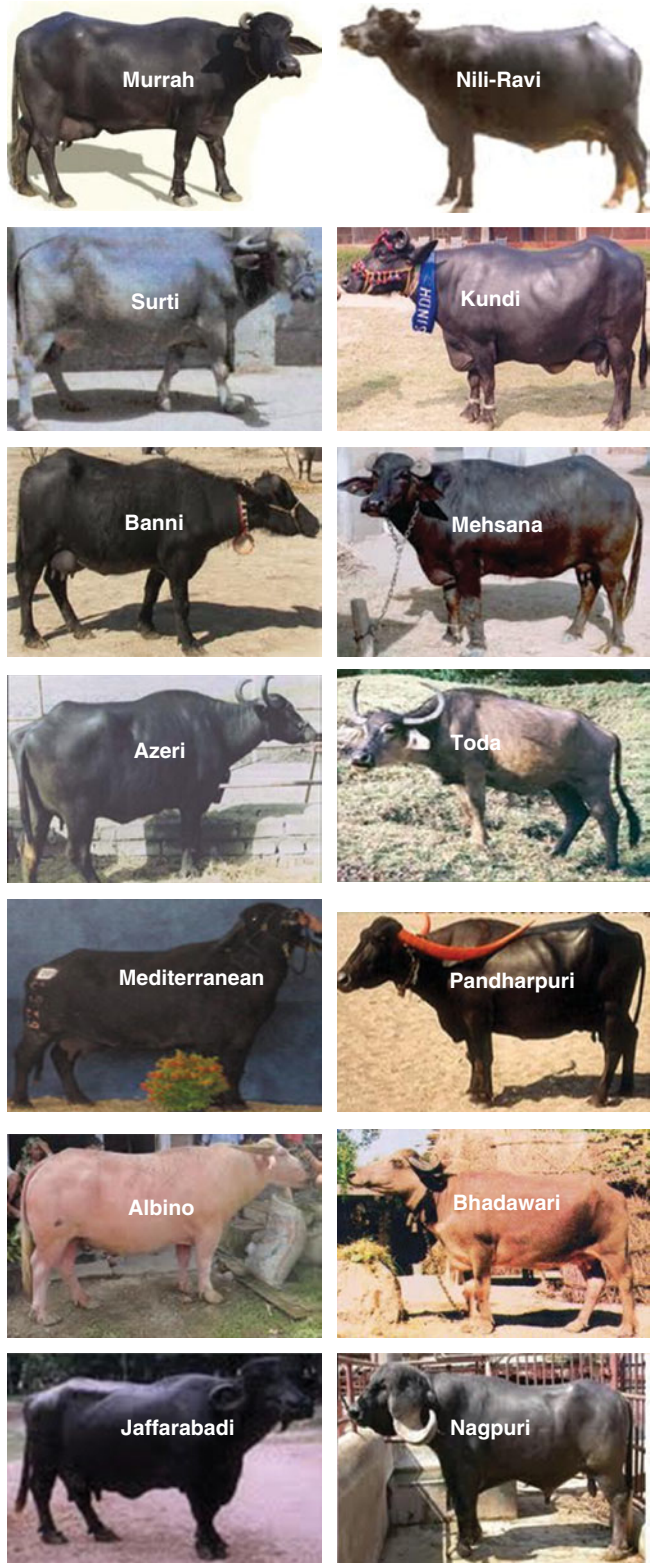


Plate 24.1. Major dairy buffalo breeds.



Plate 26.1. Domesticated Bactrian camel in Gobi desert area. (Left) A nursing camel. (Right) A camel being milked by a nomad woman. Photos taken by the author in March 2009 in Haruult and Zagiin-Us, Ulziit soum, Dundgovi aimag in Mongolia (about 260 km south of Ulaanbaatar).



Plate 26.2. A cask for making acidified camel milk. Photo taken by the author in March 2009 in the same location as for Plate 26.1.

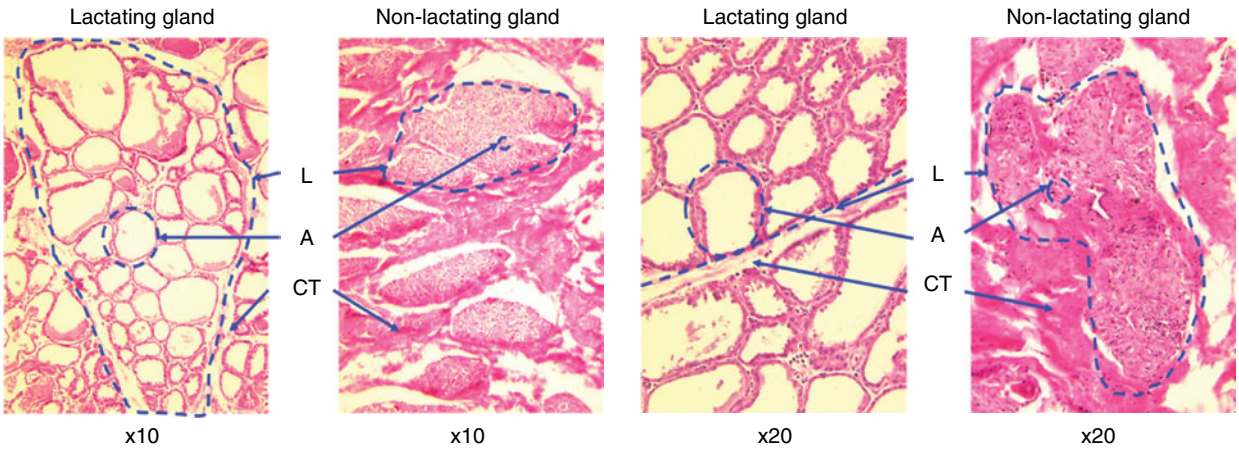


Plate 28.1. Structure of porcine mammary gland. L, lobule; A, alveoli; CT, connective tissue. (Left) ×10. (Right) ×20. Based on Kim (1999).



Plate 30.1. A milkmaid at the Kostroma Moose Farm in Kostroma Oblast, Russia, prepares to milk a moose. From http://ca.wikipedia.org/wiki/Fitxer:Milkmaid-and-Moose-Cow-hp4080.jpg. Courtesy of Dr Alexander Minaev.

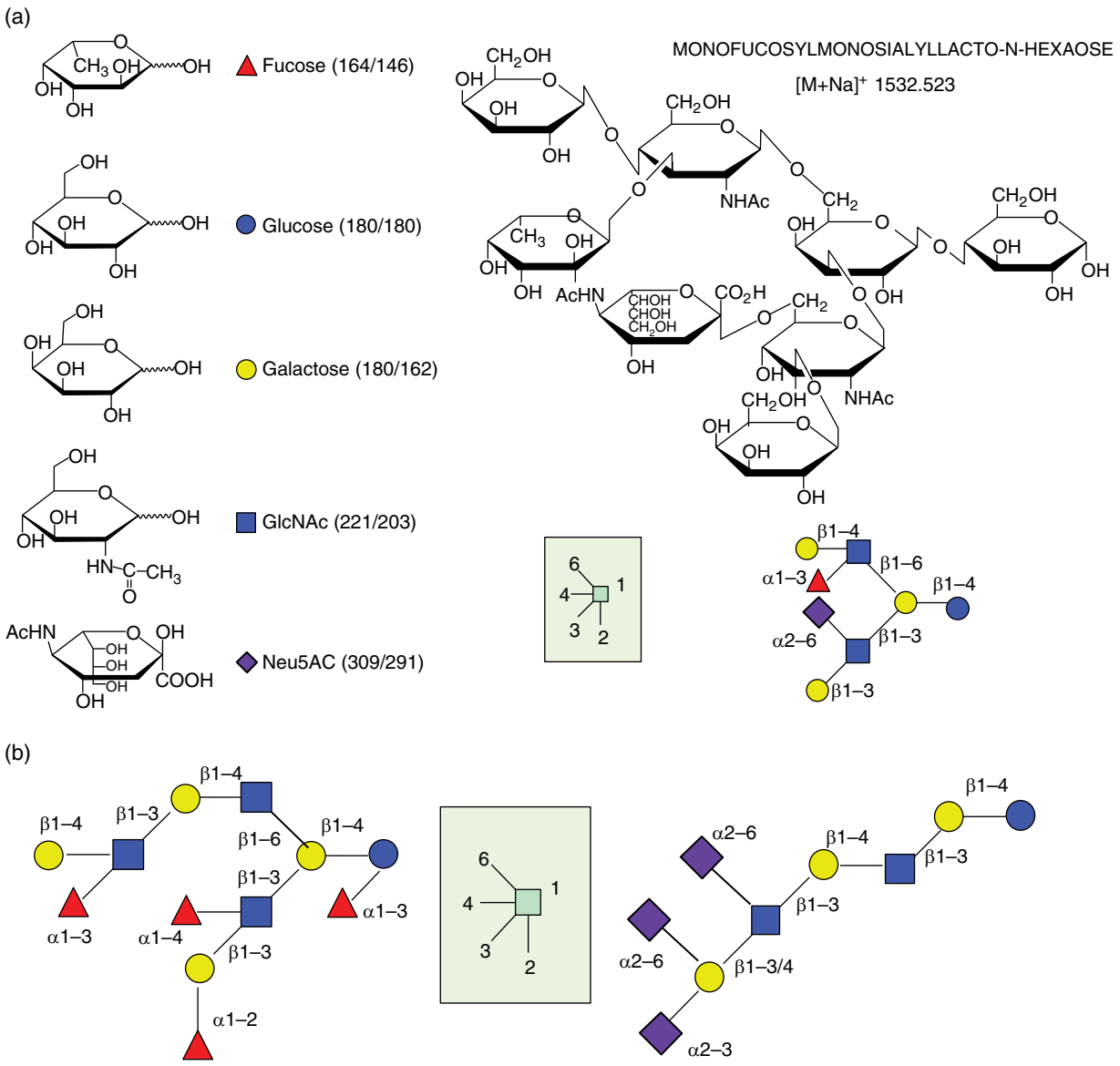


Plate 31.1. (a) Illustration of a human milk oligosaccharide (HMO) structure with key for interpreting symbols. (b) Examples of "branched" and "linear" HMO structures. Reproduced with permission from Wu *et al.* (2010). Copyright (2010) the American Chemical Society.

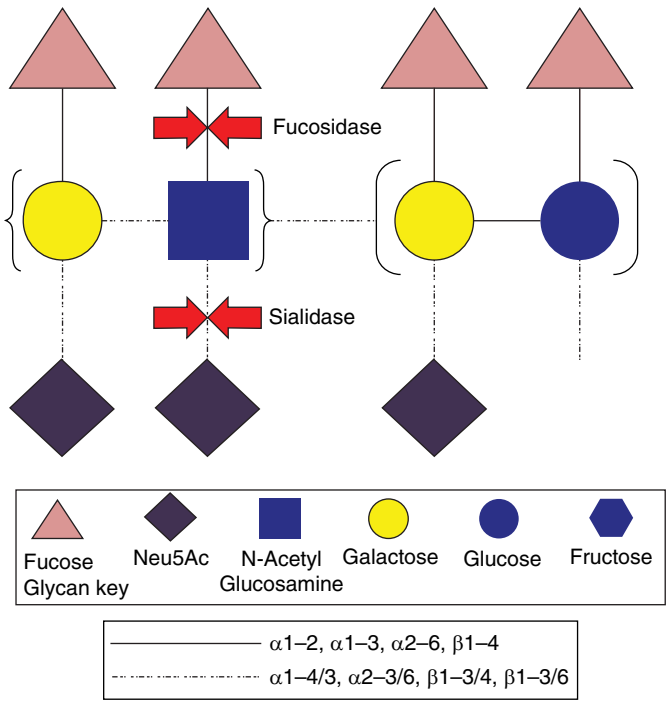


Plate 31.2. Basic structure of HMO molecules. Sites of fucosidase and sialidase activity are denoted by arrows. Reproduced from Sela (2011), with permission of Elsevier.