

Dairy Processing & Quality Assurance



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Ramesh C. Chandan, Arun Kilara and Nagendra P. Shah



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Preface

The objective of our book *Dairy Processing and Quality Assurance* is twofold. First, this book should provide an updated hands-on textbook on Dairy Food Processing for upper-level students enrolled in Food Science programs in various universities. Second objective is to provide an updated applied reference book for professionals engaged in management, quality assurance, and manufacturing in the dairy food industry.

The editorial team assembled 28 authors from the United States, Australia, New Zealand, United Kingdom, and Ireland to write the chapters. These contributors represent diverse expertise from academia, food industry, and government research institutions to insure current practical information, scientific accuracy, and potential instructional value to all engaged in the processing and quality assurance disciplines of dairy food industry. This book is not meant to be a treatise on the subject but presents basic information on the subject in a concise, easily understandable style.

This book gives a description of the processing and manufacturing stages of market milk and major dairy products from the receipt of raw materials to the packaging of the products, including quality assurance aspects. Modern quality and safety management techniques have been incorporated to appraise the reader with current trend in the field. Information is conveniently grouped under 23 chapters written by multiple authors. The individuality of authors' contribution has been retained by the editors in order to give diversity of regulatory practices prevalent in the authors' domicile. No attempt has been made to provide a comprehensive rules and regulations controlling production of dairy foods in various parts of the world. The state of dairy food industry in the United States has been discussed in first two chapters.

Chapter 1 gives an overview of the dairy industry. Chapter 2 discusses production and consumption trends in the United States. Chapter 3 deals with the fundamental information about the mammary gland of the cow and biosynthesis of milk and milk constituents. Chapters 4 and 5 describe chemical, physical, and microbiological basis of milk processing. Chapter 4 deals with chemical composition, physical structure, and functional properties of milk. Chapter 5 contains information on microbiological considerations related to milk processing. Chapter 6 discusses regulations for product standards and labeling in the United States. Chapter 7 covers steps in the transportation to the processing plant including milk storage and handling at the plant. The theme is how to assure quality and safety of milk. Chapter 8 describes some of the ingredients used in processing of dairy products. Chapters 9–17 are dedicated to processing and production of market milk and various dairy foods. Coverage includes fluid milk products (Chapter 9), cultured milk and yogurt (Chapter 10), butter and spreads (Chapter 11), cheese (Chapter 12), evaporated and condensed milk (Chapter 13), dry milk products (Chapter 14), whey and whey products (Chapter 15), ice cream and frozen desserts (Chapter 16), followed by puddings and dairy desserts (Chapter 17). The role of milk and dairy foods in human nutrition is described in Chapter 18. Strategies for new product development are given in Chapter 19. Chapter 20 is devoted to packaging milk and milk products. Nonthermal processing technologies for dairy products are discussed in Chapter 21. Chapter 22 is devoted to modern management systems for safety and quality. Chapter 23 describes a myriad of laboratory analysis techniques related to ensuring quality and safety of milk and dairy products.

In general, an attempt has been made to support manufacturing processes on sound scientific technological, and engineering principles prevalent in dairy food industry. Quality assurance procedures are given for each product at the end of the appropriate chapter. The book presents a contemporary update and a unique approach to the topics, and is designed to augment related books in the existing market. The editorial team is comprised of individuals with significant experience in the science and applications of dairy products manufacture. It is hoped that Dairy

Processing Technology and Quality Assurance will appeal to professors, extension staff, and students in dairy science for its contemporary information and experience-based applications. Also, the book should be useful for food scientists, regulatory personnel, dairy equipment manufacturers, and technical specialists in the dairy food industry.

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Dairy Processing and Quality Assurance

1

Dairy Processing and Quality Assurance: An Overview

Ramesh C. Chandan

Introduction	Enzyme-Modified Cheeses
Basic Steps in Milk Processing	Cheese Sauces
Basic Processing Steps in a Dairy Plant	Whey Products
Manufacture of Fluid Milk Products	Dry Sweet Whey
Milk	Fractionated Whey Products
Cream	Other Dry Milk Products
Concentrated Milk Fat Products	Refrigerated Dairy Desserts/Snacks
Butter	Ice Cream and Frozen Desserts
Light/Reduced Fat Butter	Soft Frozen Dairy Products
Butter Oil	Nutrient Profile of Dairy Foods
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Cold Pack Cheese Food	
Cheese Powders	

INTRODUCTION

Dairy processing involves conversion of raw milk into fluid milk products, and an array of dairy products such as butter, yogurt, and fermented milks, cheeses, dry milk powders, dry whey products, ice cream, and frozen and refrigerated desserts.

Factors related to the cow such as breed, intervals of milking, stages of milking, different quarters of udder, lactation period, season, feed, nutritional level, environmental temperature, health status, age, weather, estrus cycle, gestation period, and exercise are known to cause variations in fat, protein, lactose, and mineral levels in milk derived from individual cows. In general, these variations tend to average out and display an interesting pattern in commercial milk used by the processors. However, the seasonal variations in major milk constituents are relevant to the processor since they impact important properties of finished products. In general, in the United States, approximately 10% variation in fat and protein is

observed in milk received in July–August (lowest level) as compared to milk delivered in October–November (highest level). Subsequently, functional contribution of milk proteins (viscosity in yogurt, buttermilk as well as curd firmness in cheese manufacture) also follows similar trend. Furthermore, cheese yield and whey protein production are also negatively affected by seasonal variations in milk composition.

The concentration of minerals such as chloride, phosphates, and citrates of potassium, sodium, calcium, and magnesium in milk is important in processing, nutritive value, and shelf life of dairy products. Their concentration is <1% in milk but are involved in heat stability of milk, alcohol coagulation of milk, age-thickening of sweetened condensed milk, feathering of coffee cream, rennin coagulation, and clumping of fat globules on homogenization. All the minerals considered essential for human nutrition are found in milk (Chandan, 2007a). From consumer standpoint, quality factors associated with milk are appearance, color, and sensory attributes such as aroma, flavor, and mouth feel.

The color of milk is perceived by consumer to be indicative of purity and richness. The white color of milk is due to the scattering of reflected light by the inherent ultramicroscopic particles, fat globules, colloidal casein micelles, and calcium phosphate. The intensity of white color is directly proportional to size and number of particles in suspension. Homogenization increases the surface area of fat globules significantly as a result of breakup of larger globules. Accordingly, homogenized milk and cream are whiter than nonhomogenized counterparts. After the precipitation of casein and fat by the addition of a dilute acid or rennet, whey is separated which possesses a green–yellow color due to the pigment riboflavin. The depth of color varies with the amount of fat remaining in the whey. The lack of fat globules gives skim milk a blue tinge. Physiological disturbances in the cow make the milk bluer.

Cow's milk contains pigments carotene and xanthophylls, which tend to give golden yellow color to the milk. Guernsey and Jersey breeds produce especially golden yellow milk. Milk from goats, sheep, and water buffalo tends to be much whiter in color because their milk lacks the pigments.

The flavor (taste and aroma) of milk is critical to its assessment criterion of quality by the consumer. Flavor is an organoleptic property where both odor and taste interact. The sweet taste of lactose is balanced against the salty taste of chloride, and both are some-

what moderated by proteins. This balance is maintained over a fairly wide range of milk composition even when chloride ion varies from 0.06 to 0.12%. Saltiness can be detected organoleptically in samples containing 0.12% or more of chloride ions and becomes marked in samples containing 0.15%. Some workers attribute the characteristic rich flavor of dairy products to the lactones, methyl ketones, certain aldehydes, dimethyl sulfide and certain short chain fatty acids. As lactation advances, lactose declines while chlorides increase, so that the balance is slanted toward "salty." A similar dislocation is caused by mastitis and other udder disturbances. Accordingly, milk flavor is related to its lactose/chloride ratio.

Freshly drawn milk from any mammal possesses a faint odor of a natural scent peculiar to the animal. This is particularly true of the goat, mare, and cow. The "cow" odor of cows' milk is variable, depending upon the individual season of the year, and the hygienic conditions of milking. A strong "cowy" odor frequently observed during the winter months may be due to the entry into milk of acetone bodies from the blood of cows suffering from ketosis. Feed flavors in milk originate from feed aromas in the barn; for instance, aroma of silage. In addition, some feed flavors are imparted directly on their ingestion by the animal. Plants containing essential oils impart the flavor of the volatile constituent to the milk. Garlic odor and flavor in milk is detected even 1 minute after feeding garlic. Weed flavor of chamomile or mayweed arises from the consumption of the weed in mixtures of ryegrass and clover. Cows on fresh pasture give milk with a less well-defined "grassy" flavor, due to coumarin in the grass. A "clovery" flavor is observed when fed on clover pasture and these taints are not perceptible when dried material is fed. Prolonged ultraviolet radiation and oxidative taints lead to "mealiness," "oiliness," "tallowiness," or "cappy" odor. Traces of copper (3 ppm) exert development of metallic/oxidized taints in milk. Microbial growth in milk leads to off-flavors such as acid (sour), proteolytic (bitter), and rancid. Raw milk received at the plant should not exhibit any off-flavors. Certain minor volatile flavor could be volatilized off by dairy processing procedures.

Dairy technology may be defined as the application of theoretical and applied scientific knowledge to transforming milk into articles of commerce. Dairy processing involves chemical, microbiological, physical, and engineering principles and it is imperative to understand them for effective management of a dairy plant. Additionally, meeting consumer expectations

by controlling the processes to deliver quality, safety, and shelf life of the products is paramount to successful dairy processing operation. In the recent past, major advances in dairy processing have resulted in improvement in safety and quality of products. Such developments have led to increased sophistication in mechanization, automation, computerization, sanitation, ultra-pasteurization, and aseptic packaging in dairy plants. Research and development work undertaken at the university, government, and private industry level has further added basic and applied knowledge to dairy industry. The work has benefited consumer by making products safer and extending their shelf life for making them available over wider distribution areas. Furthermore, research and development efforts have led to the introduction of an array of new products providing a wide variety of new products in market place.

The industry continues to consolidate and make large investments in new dairy processing facilities handling significantly more volume of milk than ever before. Chapter 2 discusses the production and consumption trends in dairy industry. The consolidated plant operations have taken advantage of innovations in plant design and machinery and new systematic quality management programs like hazard analysis critical control points (HACCP) to insure product quality and safety. Developments in electronic data processing and process control are routinely practiced in many dairy plants. In addition, modern membrane technologies like ultra-filtration reverse osmosis, and electrodialysis in whey and cheese manufacture have resulted in profitable utilization of erstwhile waste streams from dairy product manufacturing. Sewage treatment facilities attached to manufacturing plants have helped in control of effluent pollution problems. Furthermore, advances in biotechnology of lactic cultures and enzymes have been adopted for optimization in cheese production and ripening as well as for efficiency in yogurt and fermented milk processes. Ultra-pasteurization techniques and aseptic packaging systems have presented the consumer with extended and long shelf-life products.

Dairy personnel are the key to the operation of a dairy plant. They make sure that raw materials of optimum quality and prescribed specification are available, stored, and utilized in a timely manner. They apply the standard analytical procedures (approved by regulatory authorities) for optimum processing and packaging, storage, and shipment of the final products. In this area, they make crucial decisions relative

to acceptance or rejection of raw materials as well as of finished products. In short, they are responsible for quality control programs for raw materials, in-process controls, and finished product specification to insure compliance with regulatory and proprietary standards. All the sensory, chemical, physical, and microbiological aspects must be met for proper functioning of the plant. In addition, approved sanitation programs and other quality systems have to be implemented for successful management of the plant.

This chapter deals with major dairy products and outlines of basic dairy processes used for making them. The details of the processes and quality assurance procedures follow in succeeding chapters of this book.

BASIC STEPS IN MILK PROCESSING

Major components of commercial raw milk are illustrated in Figure 1.1. Chapter 3 deals with the biosynthesis and origin of milk constituents. And, Chapter 4 discusses the chemical composition, physical and functional properties of milk and milk ingredients.

On dry basis, raw milk calculates to contain 28.57% fat, 26.98% protein (21.43% casein, 5.55% whey proteins), 38.89% lactose, and 5.55% ash. The composition of skim milk solids amounts to 54.44% lactose, 37.78% protein (30% casein and 7.78% whey protein), 1.11% fat, and 7.78% ash.

Milk production, transportation, and processing are regulated by Grade A pasteurized milk ordinance (USDHHS PMO, 2003). Figure 1.2 shows journey of milk from the farm to supermarket, including processing at the milk plant. Chapters 6 and 7 are dedicated to regulatory control of milk production and transportation to milk processing plant.

BASIC PROCESSING STEPS IN A DAIRY PLANT

Basic dairy processing principles are described elsewhere (Kilara, 2006). New processing technologies are enumerated in Chapter 21 and new product development strategies are given in Chapter 19.

A summary of various stages is given below.

Bulk Milk Handling and Storage

It is a key position in handling of good quality milk. Dairy farms produce sanitary raw milk under the supervision of U.S. public health services. Chapter 5 discusses the microbiological aspects of milk

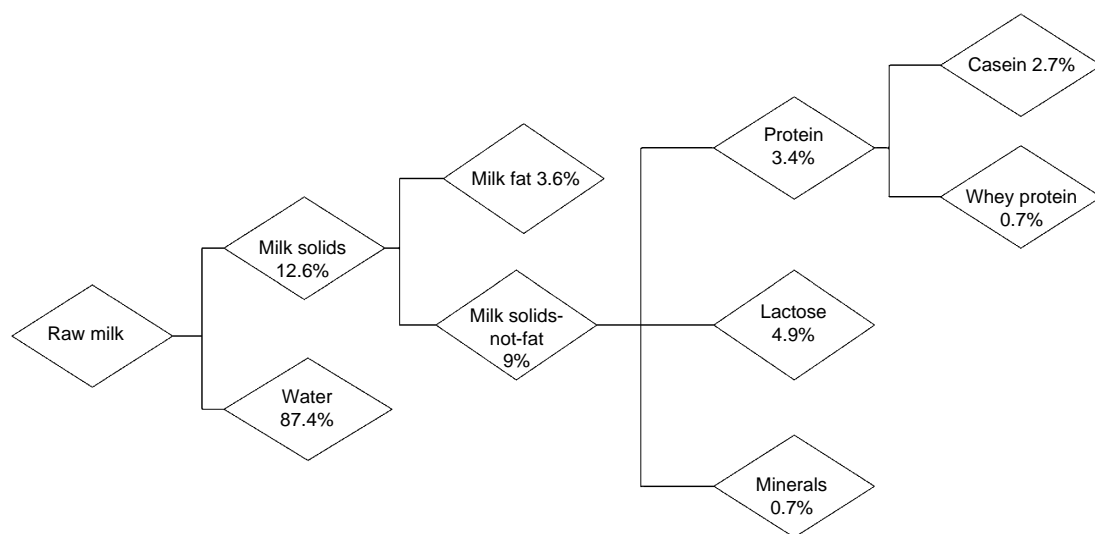


Figure 1.1. Gross composition of pooled raw milk.

processing. Chapters 6 and 7 deal with details of regulations relative to milk production, handling, and storage at the dairy processing plant. In particular, milk production under Grade A regulations protects consumers from contracting milk-borne diseases which in the past were commonly associated with contaminated milk consumption. The regulations help in the movement of assured quality milk across interstate lines.

Virtually, all the raw milk at the plant is delivered in tank trucks. Unloading of milk involves agitation of truck, inspection for the presence of off-flavors, taking a representative sample, and connecting the unloading hose to the truck outlet. After opening the tank valve, a high capacity transfer pump is used to pump milk to a storage tank or silo. The weight of milk transferred is registered with a meter or load cells. The tank truck is then cleaned by plant personnel by rinsing with water, cleaning with detergent solution, rinsing again with water followed by a chlorine/iodine sanitizing treatment. A clean-in-place line may be inserted into the tank through the manhole. Payment of milk is based on the hauler receipt.

Storage tanks may be refrigerated or insulated. They hold milk up to a period of 72 hours (usually 24 hours) before processing. The tanks may be horizontal or vertical in configuration. Grade A milk for pasteurization must be stored at 1.7–4.4°C (35–40°F). Maximum bacterial count is 300,000 CFU/mL as

opposed to 100,000 CFU/mL, the maximum allowed at the farm. The higher count is justified because pumping breaks the clumps of bacteria giving higher counts and there is more opportunity of contamination of milk as it comes in contact with more equipment during handling and transfer. Also, longer time of storage adds more bacterial numbers. For equipment design, the 3-A sanitary standards are followed (Frye, 2006).

Separation

The purpose of this step is to separate milk into cream and skim milk. All incoming raw milk is passed through separators, which are essentially high-speed centrifuges. They separate milk into lighter cream fraction and heavier skim milk fraction. A separator of adequate bowl capacity should collect all the slime material containing heavy casein particles, leukocytes, larger bacteria, body cells from cow's udder, dust and dirt particles, and hair. If the particulate fraction of raw milk is not removed, homogenized milk will develop sediment upon storage. Skim milk and cream are stored separately for further processing.

Standardization

Use of a separator also permits fractionation of whole milk into standardized milk (or skim milk, low-fat

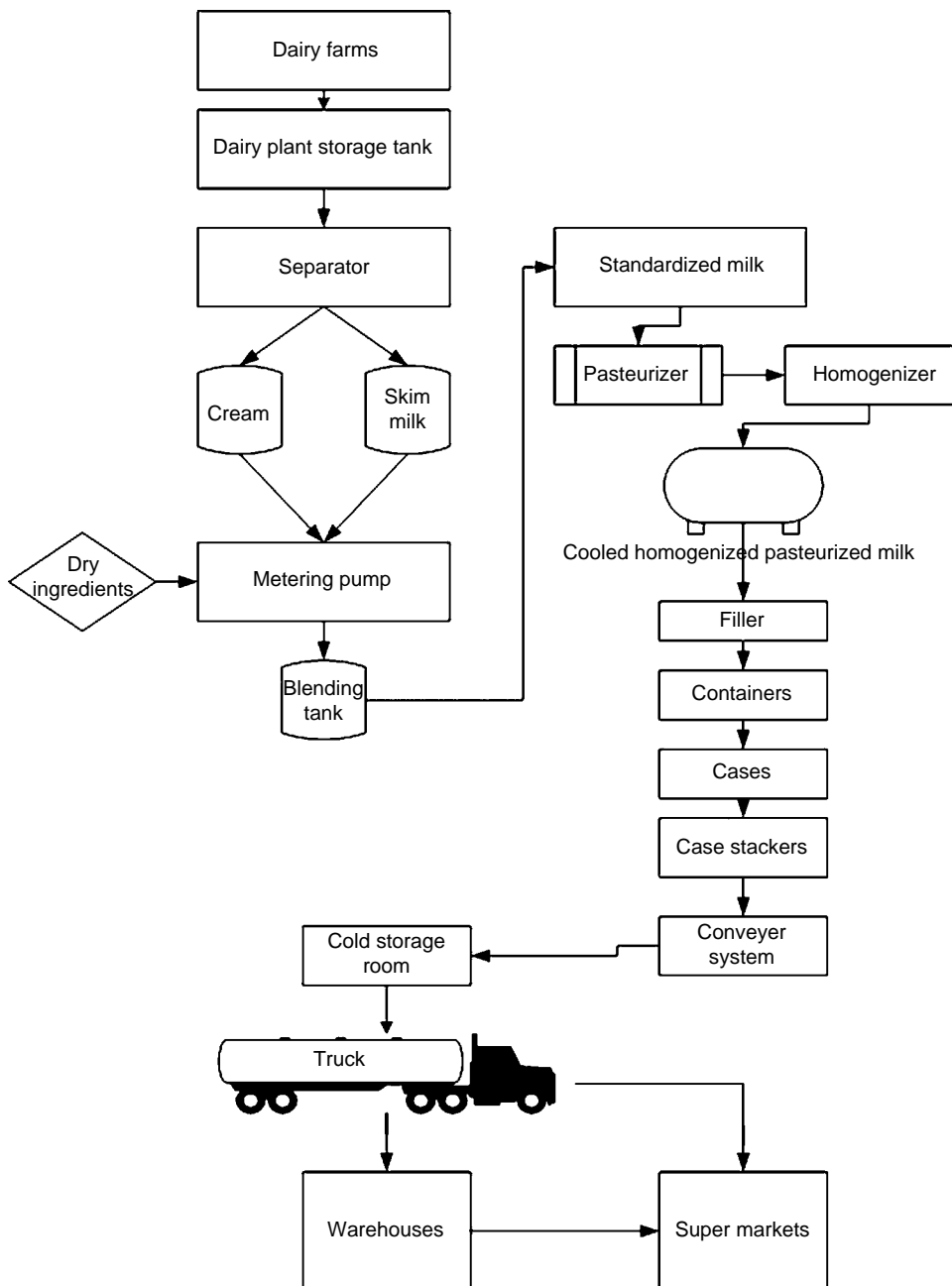


Figure 1.2. Journey of milk from farms to supermarkets.

milk) and cream. Skim milk should normally contain 0.01% fat or less. Standardization valve on the separator permits the operator to get separated milk of predetermined fat content. Increased back pressure

on cream discharge port will increase fat content in standardized milk. By blending cream and skim milk fractions, various fluid milk and cream products of required milk fat content can be produced.

Table 1.1. Minimum Time–Temperature Requirements for Legal Pasteurization in Dairy Operations

Process	Milk—Whole Low-Fat, Skim/Nonfat	Milk Products with Increased Viscosity, Added Sweetener, or Fat Content 10% or More	Eggnog, Frozen Dessert Mixes
Vat (batch)	30 minutes at 63°C (145°F)	30 minutes at 65.6°C (150°F)	30 minutes at 69°C (155°F)
High temperature, short time	15 seconds at 72°C (161°F)	15 seconds at 74.4°C (166°F)	25 seconds at 80°C (175°F) 15 seconds at 83°C (180°F)
Higher heat, shorter time	1.0 second at 89°C (191°F) 0.5 second at 90°C (194°F) 0.1 second at 94°C (201°F) 0.05 second at 96°C (204°F) 0.01 second at 100°C (212°F)	1.0 second at 89°C (191°F) 0.5 second at 90°C (194°F) 0.1 second at 94°C (201°F) 0.05 second at 96°C (204°F) 0.01 second at 100°C (212°F)	1.0 second at 89°C (191°F) 0.5 second at 90°C (194°F) 0.1 second at 94°C (201°F) 0.05 second at 96°C (204°F) 0.01 second at 100°C (212°F)
Ultra-pasteurized	2.0 seconds at 137.8°C (280°F)	2.0 seconds at 137.8°C (280°F)	2.0 seconds at 137.8°C (280°F)

Heat Treatment

The main purpose of heat treatment of milk is to kill all the disease-producing (pathogenic) organisms and to enhance its shelf life by removing approximately 95% of all the contaminating organisms. Heat treatment is an integral part of all processes used in dairy manufacturing plants. Intensive heat treatment brings about interactions of certain amino acids with lactose resulting in color changes in milk (Maillard browning) as observed in sterilized milk and evaporated milk products. Among milk proteins, caseins are relatively stable to heat effects. Whey proteins tend to denature progressively by severity of heat treatment, reaching 100% denaturation at 100°C (212°F). In the presence of casein in milk, denatured whey proteins complex with casein and no precipitation is observed. In contrast to milk, whey that lacks casein, heat treatment at 75–80°C (167–176°F) results in precipitation of the whey proteins. From a consumer standpoint, heat treatment of milk generates several sensory changes (cooked fl vor) depending on intensity of heat. In general, pasteurized milk possesses the most acceptable fl vor. Ultra-pasteurized

milk and UHT milk exhibit slightly cooked fl vor. Sterilized milk and evaporated milk possess exceedingly cooked fl vor and off-color.

The U.S. Food and Drug Administration have define pasteurization time and temperature for various products. The process is regulated to assure public health. Using plate heat exchangers with a regeneration system, milk is pasteurized to render it free of all pathogenic organisms and to reduce 95% of other microbial load. The process of pasteurization involves heating every particle of milk or milk product in properly designed and operated equipment to a prescribed temperature and held continuously at or above that temperature for at least the corresponding specific time. Minimum temperature–time requirements for pasteurization are based on thermal death time studies on the most resistant pathogen that might be transmitted through milk. Table 1.1 shows the various time–temperatures for legal pasteurization of dairy products.

Most refrigerated cream products are now ultra-pasteurized by heating to 125–137.8°C (257–280°F)

for 2–5 seconds and packaged in sterile cartons in clean atmosphere. Milk for ambient storage is UHT treated at 135–148.9°C (275–300°F) for 4–15 seconds, followed by aseptic packaging. In some countries, sterilized/canned milk is produced by sterilizing treatment of 115.6°C (240°F) for 20 minutes. It has a brown color and a caramelized flavor.

Homogenization

This process reduces the size of fat globules of milk by pumping milk at high pressure through a small orifice called valve. The device for size reduction is called a homogenizer which subjects fat particles to a combination of turbulence and cavitation. Homogenization is carried out at temperatures higher than 37°C (99°F). The process causes splitting of original fat globules (average diameter approximately 3.5 μm) into a very large number of much smaller fat globules (average size <1 μm). As a consequence, a significant increase in surface area is generated. The surface of the newly generated fat globules is then covered by new membrane formed from milk proteins. Thus, the presence of a minimum value of 0.2 g of casein/g fat is desirable to form to coat the newly generated surface area. As milk is pumped under high-pressure conditions, the pressure drops causing breakup of fat particles. If the pressure drop is engineered over a single valve, the homogenizer is deemed to be single-stage homogenizer. It works well with low-fat products or in products where high viscosity is desired as in creams and sour cream manufacture. On the other hand, homogenizers reducing fat globule size in two stages are called dual-stage homogenizers. In the first stage, the product is subjected to high pressure, for example, 13.8 MPa (2,000

psi) which results in break down of the particle size diameter to an average of less than 1 μm . Then the product goes through the second stage of 3.5 MPa (500 psi) to break the clusters of globules formed in the first stage. The dual-stage homogenization is appropriate for fluid with high-fat and solids-not-fat content or whenever low viscosity is needed.

Homogenized milk does not form a cream layer (creaming) on storage. It displays whiter color, fuller body, and flavor characteristics. Homogenization leads to better viscosity and stability in cultured products by fully dispersing stabilizers and other ingredients in ice cream, yogurt, and other formulated dairy products.

Cooling, Packaging, and Storage

The pasteurized fluid milk products are rapidly cooled to less than 4.4°C/40°F, packaged in appropriate plastic bottles/paper cartons and stored in cold refrigerated rooms for delivery to grocery stores or warehouse for distribution. Chapter 20 gives a detailed description of packaging materials and machinery.

MANUFACTURE OF FLUID MILK PRODUCTS

Dairy products may be classified as fluid milk products, butter and butter products, concentrated and dry milk products, cultured milk products, cheese products, whey products, and ice cream and frozen desserts.

Approximate composition of fluid milk products is shown in Table 1.2. Chapter 9 of this book deals with the fluid milk and milk products.

Table 1.2. Typical Composition of Fluid Milk, Cream, and Fluid Dairy Ingredients

Dairy Ingredient	% Water	% Fat	% Protein	% Lactose	% Ash
Whole milk	87.4	3.8	3.2	4.9	0.7
Skim milk	90.9	0.1	3.3	5.0	0.7
Half and half	80.2	11.5	3.1	4.5	0.7
Light cream	74.0	18.3	2.9	4.2	0.6
Light whipping cream	62.9	30.5	2.5	3.6	0.5
Heavy whipping cream	57.3	36.8	2.2	3.2	0.5
Plastic cream	18.2	80.0	0.7	1.0	0.1
Fluid UF whole milk	70–75	11–14	10–12	<5	>2.5
Fluid UF skim milk	80–85	<0.5	10–12	<5	>2.5
Fluid UF skim milk, diafiltered	80–82	<0.5	<16–17	<1	>1.5

UF, ultrafiltered

Adapted from Chandan (1997) and Chandan and O'Rell (2006a).

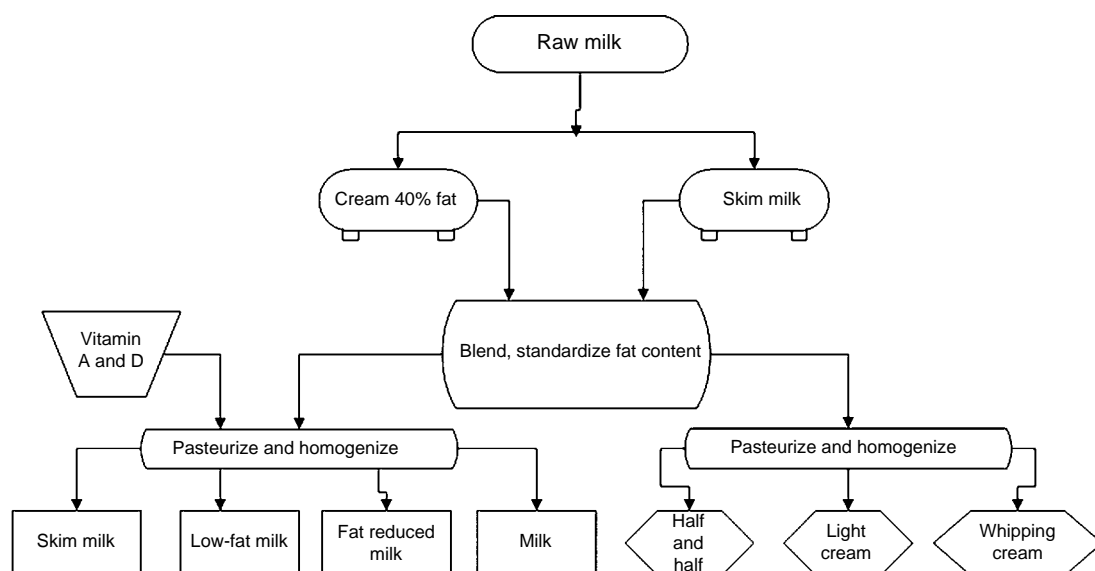


Figure 1.3. Fluid milk processing flow sheet.

MILK

Commercial milk is available in various milk fat contents. The term “milk” is synonymous with whole milk, which must contain not less than 3.25% milk fat and 8.25% solids-not-fat. Addition of vitamins A and D is optional. If the vitamins are added, vitamin A must be present at a level of not less than 2,000 International Units (IU)/quart and vitamin D must be present at 400 IU/quart of milk.

Fat-reduced milks are labeled according to their contribution of grams of fat per reference amount (RA) of 240 mL. Low-fat milk contributes less than 3 g fat per RA, while nonfat milk contributes less than 0.5 g of fat per RA. Accordingly, milk containing 2% milk fat does not qualify to be labeled as low-fat milk and is labeled reduced-fat milk. Low-fat milk available in the market place has 1% milk fat and nonfat/fat free/skim milk can legally have 0.2% fat. However, in reality nonfat milk contains less than 0.1% fat. All the fluid milk beverages may have added vitamins A and D.

Figure 1.3 shows the steps in production of fluid milk and cream products.

The figure shows general processes for manufacture of whole milk, reduced fat milk, low-fat milk, and skim milk. It also shows how cream and other fluid products are made. Normally, whipping cream is not homogenized.

The shelf life of milk is a function of the microbial quality of raw milk, temperature and time exposure during storage and handling, pasteurization conditions, equipment sanitation, packaging conditions, and subsequent distribution practices. The shelf life of milk purchased from grocery stores is dependent largely on the storage temperature. Fluid milk products display maximum shelf when stored at temperature close to freezing point ($4^{\circ}\text{C}/32^{\circ}\text{F}$). Let us assume the shelf life of pasteurized milk is 40 days at storage temperature of $0^{\circ}\text{C}/32^{\circ}\text{F}$. It has been demonstrated that the shelf life gets shortened to 20 days by storage at $2^{\circ}\text{C}/35.6^{\circ}\text{F}$, 10 days at $4^{\circ}\text{C}/39.2^{\circ}\text{F}$, 5 days at $7^{\circ}\text{C}/44.6^{\circ}\text{F}$, and progressively to fewer days at higher temperatures. This illustration underscores the importance of maintaining refrigerated storage temperature as low as possible to achieve maximum shelf life of milk.

Ultra-pasteurized products are packaged in a near-aseptic atmosphere in presterilized containers and held refrigerated to achieve an extended shelf life. When ultra-pasteurized product is packaged aseptically in specially designed multilayer container, it displays shelf life even longer than any other packaged fluid milk and cream products.

UHT products subjected to aseptic heat treatment and aseptically packaged in specially designed containers can be stored at ambient temperatures for several months.

Table 1.3. Typical Composition of Milk Fat Concentrates

Product	% Water	% Fat	% Protein	% Lactose	% Ash	Added Ingredient
Butter	16.5	80.5	0.6	0.4	2.5	0–2.3% Salt
Anhydrous milk fat	0.1	99.8	0.1	0	0	0
Butter oil	0.3	99.6	0.1	0	0	0
Ghee	<0.5	99–99.5	0	0	0	0

Adapted from Chandan (1997) and Aneja et al. (2002).

CREAM

Cream is prepared from milk by centrifugal separation. Heavy cream contains not less than 36% fat and may be called “heavy whipping” cream. Light whipping cream contains 30% or more milk fat, but less than 36% milk fat and may be labeled as whipping cream. Light cream, coffee cream, or table cream contains not less than 18% milk fat, but less than 30% milk fat. Half and half is normally a blend of equal proportion of milk and cream, containing 10.5% milk fat. Legally, it contains not less than 10.5% milk fat but not more than 18% milk fat. Cream to be used as an ingredient in processing contains 36–40% fat. By standardizing with skim milk, cream of different fat levels can be produced. Light cream, and half and half are homogenized products. Specific homogenization and heat treatments generate desirable grades of viscosity in cream products. They are processed and packaged similar to fluid milks.

Plastic cream contains 80% milk fat. It resembles butter in consistency but compared to butter, it is still oil-in-water type emulsion. It can be stored in frozen form.

Milk and other dairy products are used as ingredients in various food products. They perform an important nutritional and functional role. The functional properties of dairy ingredients are related to their chemical composition and specific processing conditions to which they may be subjected in order to modify their performance in a given food system. Chapter 8 gives detailed information on dairy ingredients.

CONCENTRATED MILK FAT PRODUCTS

BUTTER

Butter is a concentrated form of milk fat, containing at least 80% fat. It can be converted to shelf stable products such as butter oil, anhydrous milk fat, and

ghee. Table 1.3 shows the approximate composition of butter and its products.

Figure 1.4 shows a flow sheet diagram for the manufacture of butter, butter oil, and certain dry milk products. The diagram also displays interrelationships between these products.

Butter is obtained by churning of cream. The temperature of churning is an important parameter to follow. The churning temperature is determined by an optimum ratio of crystalline fat, solid fat, and liquid fat. The churns are either batch type or continuous type. For batch-type churns, cream of 35–45% fat is used. For continuous-type churns, cream of 42–44% fat is used. Cream is pasteurized at 73.8°C (165°F) for 30 minutes or at 85°C (185°F) for 15 seconds and is then cooled to about 7°C (45°F) for crystallization of fat. The crystallization process is completed by holding the cream for approximately 16 hours. The cream that registers an increase in temperature to 10°C (50°F) is then transferred to sanitized churn. Annatto coloring may be incorporated, if required. The churn is rotated to convert oil-in-water type of emulsion (cream) to water-in-oil type of emulsion (butter). This conversion is known as phase inversion. This is accompanied by the appearance of butter granules of the size of popcorn or peas. During phase inversion, cream starts foaming. Free fat is generated by rupture of fat globules of cream, which cements some of the remaining fat globules to form clumps or butter granules. There is a clear separation of butter granules and surrounding liquid called buttermilk. At this stage, the buttermilk is drained out, followed by the addition of an aliquot of clean cold water (1–2°C/33.8–35.6°F) to the churn. The total volume of wash water is equal to the volume of buttermilk. The washing continues until the rinse is almost clear. Salt at 1.6% level is added and blended with butter. The next step is called “working” in which the remaining fat globules are disrupted to liberate free fat. All the free fat then forms the continuous phases in which water droplets are dispersed to form butter. Working of butter is accomplished by continuing rotation of

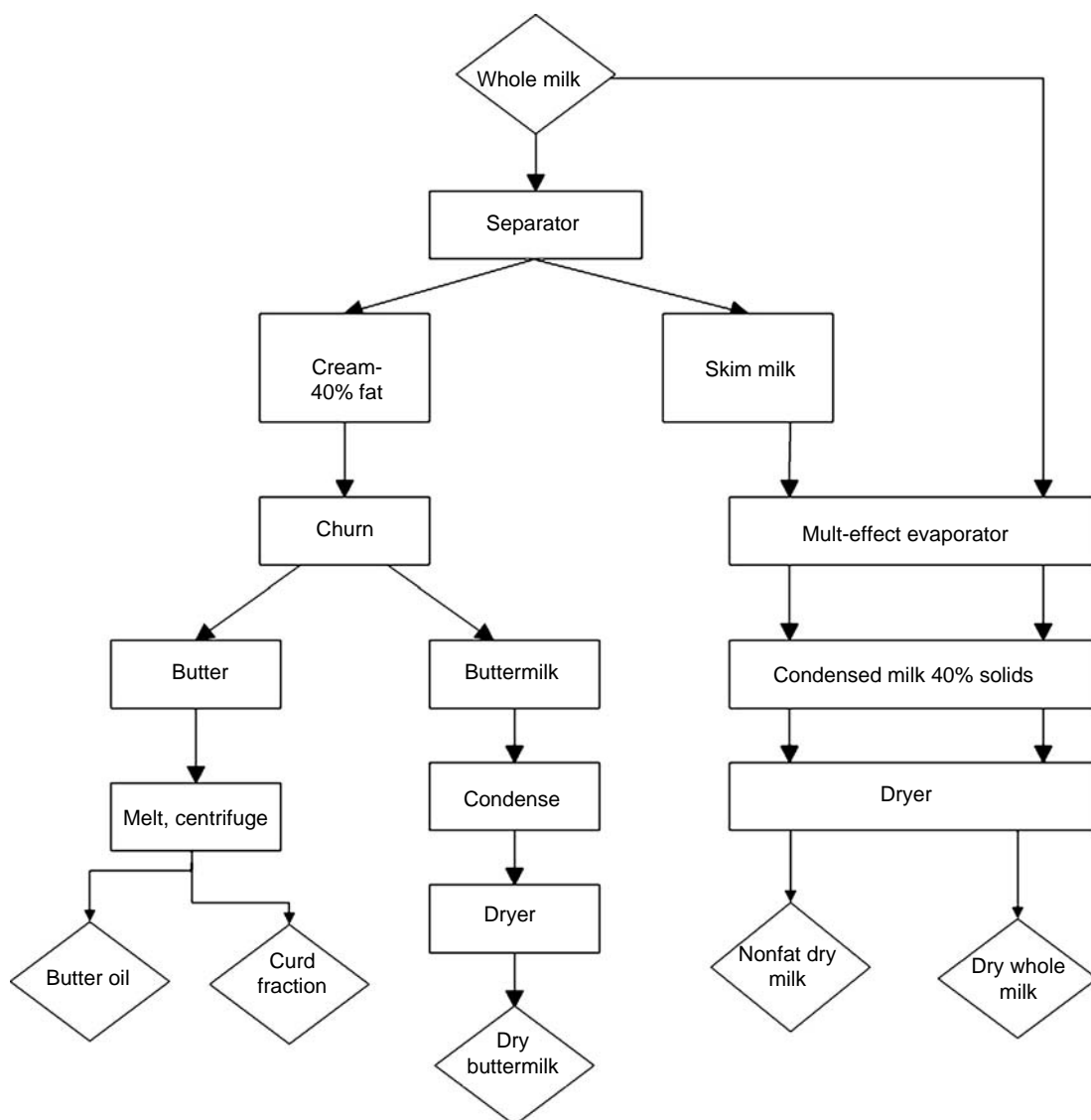


Figure 1.4. A flow sheet for the manufacture of butter, butter oil, dry buttermilk, nonfat dry milk, and whole milk powder.

the churn until the body of butter is closely knit to show a waxy character with no visible pockets of surface moisture. The “working” of butter is continued to standardize moisture until fat content of butter is 80%. Butter is then pumped and packaged.

Continuous butter churns are now widely in use. They accelerate churning process and washing of butter is not necessary. Cream of 42–44% fat is introduced into a cylinder where it is churned.

Buttermilk is drained; butter granules are worked to obtain the typical waxy body and texture of butter, and packaged. In another process, cream is separated to get plastic cream of 80% fat. The phase inversion is carried out by chilling. The butter granules are worked to achieve typical butter body and texture.

In some countries, butter is churned from cultured cream. Cultured cream butter has a distinct flavor

and can be easily distinguished from sweet cream butter.

The processing conditions affect the physical properties such as crystallization and melting behavior of butterfat. The crystal formation is mediated by nucleus formation and subsequent growth of crystals. The size of crystals is dependent on rate of crystallization. Melting behavior influence the application of butter in food products. The rate of transformation of solid fat fraction into liquid milk fat is important and is characterized by melting point range, thermal profile and solid fat content. Melting point temperature is the temperature at which milk fat melts completely to a clear liquid. It occurs at a range of 32–36°C (90–97°F) and assumes completely liquid state at 40°C (104°F) and completely solid state at –75°C (–103°F). At ambient temperature, it is a mixture of crystals and liquid phases. By manipulating temperature, butterfat has been fractionated into three fractions exhibiting distinct functionalities. Low-melting fraction melts below 10°C (50°F), middle-melting fraction melts between 10 and 20°C (50–68°F), and high-melting fraction melts above 20°C (68°F). Low-melt fraction contains significantl lower levels of saturated fatty acids. Butter made with very low-melt fraction spreads at refrigerated temperature. Further fractionation leads to very high-melting fraction that melts at >50°C (>122°F), behaving like cocoa butter in confectionery products.

LIGHT/REDUCED FAT BUTTER

Light/reduced fat butter contains 40% fat. The reduced fat form cannot be used for baking. Other spreads may contain a blend of milk fat and vegetable fat.

Chapter 11 of this book gives detailed information on butter and spreads.

BUTTER OIL

Butter oil is at least 99.6% fat and contains <0.3% moisture, and traces of milk solids-not-fat. Butter is melted by heating gently to break the emulsion and centrifuged in a special separator to collect milk fat, followed by vacuum drying.

ANHYDROUS MILK FAT

Anhydrous milk fat or anhydrous butter oil is obtained from plastic cream of 70–80% fat. Phase inversion takes place in a special unit (separator) and

the moisture is removed by vacuum drying. It contains at least 99.8% milk fat and no more than 0.1% moisture.

GHEE

Ghee is another concentrated milk fat which is widely used in tropical regions of the world, especially in South Asian countries. It is a clarified butterfat obtained by desiccation of butter at 105–110°C (221–230°F). The intense heat treatment generates characteristic aroma and flavor brought about by complex interactions of components of milk solids of butter. Detailed manufacturing procedure is given elsewhere (Aneja et al., 2002).

CONCENTRATED/CONDENSED FLUID MILK PRODUCTS

An outline for manufacturing dry whole milk, nonfat dry milk, and dry buttermilk powder is also given in Figure 1.4. For detailed description of these products, the reader is referred to Chapter 14. The functional properties of concentrated milk products including nonfat dry milk can be manipulated by specific heat treatment. It also affects the keeping quality of whole milk powder. The temperature and time combinations can vary widely depending on the required functional properties. Invariably, the milk for manufacture of concentrated milk products is pasteurized (high temperature, short time) by heating to at least 161°F (72°C), and holding at or above this temperature for at least 15 seconds. An equivalent temperature/time combination can be used. With condensed milk and nonfat dry milk, the extent of heat treatment can be measured by the whey protein nitrogen index, which measures the amount of undenatured whey protein.

Removal of a significant portion of water from milk yields a series of dairy ingredients. Consequently, these ingredients offer tangible savings in costs associated with storage capacity, handling, packaging, and transportation.

The composition of concentrated milk products is shown in Table 1.4.

CONCENTRATED MILK OR CONDENSED WHOLE MILK

Concentrated milk or condensed whole milk is obtained by removal of water from milk and contains at least 7.5% milk fat and 25.5% milk solids. Condensed milk is available in whole milk, low-fat, and

Table 1.4. Typical Composition of Condensed Milk Products

Products	% Water	% Fat	% Protein	% Lactose	% Ash	Added ingredient
Sweetened condensed whole milk	26.1	8.7	7.9	11.3	1.8	44.2% Sucrose
Sweetened condensed skim milk	28.4	0.3	10.0	16.3	2.3	42.7% Sucrose
Condensed whole milk	74.5	7.5	6.2	9.4	1.6	
Condensed skim milk (medium solids)	70.0	0.4	10.8	15.5	2.3	
Condensed skim milk (high solids)	59.9	0.4	14.4	22.3	3.0	
Evaporated whole milk	74.0	7.6	6.8	10.0	1.6	
Evaporated low-fat milk	75.2	4.0	7.6	11.4	1.8	

Adapted from Chandan (1997).

nonfat varieties. Condensed whole milk is purchased largely by confectionary industries. It is pasteurized but not sterilized by heat. It may be homogenized and supplemented with vitamin D. Chapter 13 gives detailed description of condensed and evaporated milk.

CONDENSED SKIM MILK

Condensed skim milk is commonly used as a source of milk solids in dairy applications and in the manufacture of ice cream, frozen yogurt, and other frozen desserts. Condensed milks are generally of customized orders. User plants specify total solids concentration, fat level, heat treatment, and processing conditions. The dairy concentrates offer economies of transportation costs and storage space. They have to be transported and stored at 4.4°C (40°F), and used within five days to preserve quality.

Depending on the end user requirements, raw milk is standardized to desired milk fat and nonfat solids ratio. In general, the original milk volume is reduced to about one third to yield about 25–40% solids in the final product. The standardized milk is preheated to 93.3°C (200°F) and held for 10–20 minutes. The objective of preheat treatment is to destroy microorganisms and enzymes, and to increase heat stability of milk. In addition, the viscosity of condensed milk is controlled by time–temperature regime during preheat treatment. The heated milk is concentrated in energy-efficient multieffect evaporators that operate in high-vacuum condition to boil off water at moderate temperatures of 46.1–54.4°C (115–130°F). The concentrated milk is continuously separated from

water vapor to achieve desirable concentration of milk solids. It may be homogenized prior to cooling and packaging or pumped to insulated trucks for transportation to user plants.

SWEETENED CONDENSED MILK

Sweetened condensed milk contains 60% sugar in the water phase, which imparts a preservative effect. Consequently, it has enhanced shelf life. When packaged properly, the product is stable for many months at ambient storage temperature. Since it does not need high-heat treatment for sterilization, it possesses a much better color and flavor than evaporated milk. Condensed milk may be low-fat and nonfat variety. It is derived from milk after the removal of 60% of its water. It must contain at least 8% milk fat and 28% milk solids. The viscosity of the product is high, approximating 1,000 times that of milk. Sweetened condensed milk is used in confectionery manufacture as well in the manufacture of exotic pies and desserts.

Manufacture of sweetened condensed milk resembles the manufacture of condensed skim milk given above. The addition of sugar and control of lactose crystal size require special processing procedure. The standardized milk is preheated at 135°C (275°F) for 5 seconds or 110–120°C (230–248°F) for 10–20 seconds. The ultraheat treatment is preferred over high temperature–short time treatment because it leads to lower viscosity in sweetened condensed milk. Following homogenization at 70°C (158°F) at 3.5 MPa (500 psi), milk is concentrated in an efficient evaporator at 82.2°C (180°F) and liquid sucrose is blended.

Table 1.5. Typical Composition of Dry Milk Products

Products	% Water	% Fat	% Protein	% Lactose	% Ash
Dried whole milk	3.0	27.5	26.4	37.2	5.9
Nonfat dry milk	3.2	0.8	36.0	52.0	8.0
Dried butter milk	3.0	5.3	32.4	51.3	8.0
Spray-dried cream (from 20% cream)	0.6	71.1	11.1	14.7	2.5

Adapted from Chandan (1997) and Chandan and O'Rell (2006a).

At this stage, the mix is standardized to 8.5% fat, 20% nonfat solids, and 44% sucrose. The blend is then pasteurized at 82.2°C (180°F) for 30 seconds and further standardized to desirable solids in the finishing pan. The product is cooled to 60°C (140°F), followed by seeding with finely ground lactose at the rate of 0.03% (dry matter basis). At this stage, the mixture is agitated vigorously while cooling to 18.3°C (65°F). The lactose crystal must be less than 10 μm to avoid settling in storage as well as sandiness in the product. Sweetened condensed milk is packaged in metal or plastic containers and sealed. For bulk sales, it is pumped into insulated trucks for transport and delivery to user plants.

EVAPORATED MILK

Evaporated milk is also concentrated milk that is homogenized and heat sterilized in sealed cans or bottles. It is made by boiling off 60% of the water content of milk. It must contain at least 6.5% milk fat, 16.5% nonfat milk solids, and 23% milk solids. Evaporated milk is heat-sterilized. The sterilization process renders the product safe for consumption and can be stored at room temperature for several months without deterioration of flavor. Current processing trend is to subject the product to ultraheat treatment, followed by aseptic packaging. This process gives a better color and flavor in the product than the in-can sterilized product. Typically, the concentration factor is of the order of 2.1 \times , giving milk fat level of approximately 8% and nonfat solids of approximately 18%. Low-fat evaporated milk composition is 4% fat and 20% nonfat solids, while nonfat evaporated milk contains 0.1% fat and 22% nonfat solids. Evaporated milk is mainly a retail canned product used by the consumer as a convenience ingredient in the preparation of meals, snacks, and desserts.

Manufacture of evaporated milk involves standardization of milk to desired fat to nonfat solids ratio and

preheating to 135°C (275°F) for 30 seconds. The milk is concentrated in vacuum evaporator at 68.3°C (155–180°F) and homogenized at 65°C (149°F) and 20.7 MPa (3,000 psi), first stage and 3.5 MPa (500 psi), second stage. It is then cooled to 10°C (50°F) and stabilized with disodium hydrogen phosphate to reduce age thickening during subsequent storage. The product is packaged in metal cans and sealed, followed by sterilization at 120°C (248°F) for 15 minutes. In a more recent process, the product is vacuum-concentrated and stabilized with disodium hydrogen phosphate as in the conventional process. It is then sterilized at 140.6°C (285°F) for 15 seconds, cooled to 60°C (140°F), and homogenized at 41.3 MPa (6,000 psi). After cooling to 10°C (50°F), evaporated milk is packaged aseptically in appropriate containers.

DRY MILK PRODUCTS

Table 1.5 shows typical composition of dry milk products.

NONFAT DRY MILK

Nonfat dry milk is the product resulting from the removal of fat and water from milk and contains the lactose, milk proteins, and milk minerals in the same relative proportions as in the fresh milk from which it was made. It contains not over 5% by weight of moisture. The fat content is not over 1.5% by weight unless otherwise indicated. Nonfat dry milk is utilized in dairy products, bakery, dry mixes, chemicals, meat processing, and in homes for cooking. Nonfat dry milk is manufactured by spray drying of condensed skim milk.

Spray drying involves atomizing concentrated milk into a hot air stream 356–392°F (180–200°C). The atomizer may be either a pressure nozzle or a centrifugal disc. By controlling the size of the droplets,

the air temperature, and the airflow, it is possible to evaporate almost all the moisture while exposing the solids to relatively low temperatures. Spray drying yields concentrated and dry milk ingredients with excellent solubility, flavor, and color.

The spray drying process is typically a two-stage process that involves the spray dryer at the first stage with a static fluid bed integrated in the base of the drying chamber. The second stage is an external vibrating fluid bed.

Product is moved through the two-stage process quickly to prevent overheating of the powder. Powder leaves the dryer and enters a system of cyclones that simultaneously cools it.

Roller drying is another process which is currently not widely used in the manufacture of most dry milk products. This process involves direct contact of a layer of concentrated milk with the hot surface of rotating rollers. This process causes adverse effects of excessive heat on milk components. In this process, heat often causes irreversible changes such as lactose caramelization, Maillard reaction, and protein denaturation. Roller drying typically results in more scorched powder particles and poorer powder solubility than spray drying. However, roller-dried milk absorbs more moisture than spray-dried powder and is preferred in some food applications such as bakery products.

Instant nonfat dry milk is a processed nonfat dry milk to improve its dispersion properties. It reconstitutes readily in cold water. Instantizing process involves agglomeration, a process of increasing the amount of air incorporated between powder particles. In one process, a small amount of moisture is incorporated in dry milk particles, forming porous aggregates followed by redrying, and grinding the agglomerated particles. The process results in dry milk with improved reconstitution properties. During reconstitution, the air is replaced by water and incorporated air enables a larger amount of water to come into contact immediately with the powder particles.

DRY WHOLE MILK

Dry whole milk is the product resulting from the removal of water from milk and contains not less than 26%, nor more than 40% milk fat and not more than 5.0% moisture (as determined by weight of moisture on a milk solids-not-fat basis). It is manufactured by spray drying of whole milk with added wetting agent, lecithin.

Reconstituted extra grade whole milk powder possesses sweet, pleasant flavor. It may have a slight degree of feed flavor, a definite degree of cooked flavor, and no off-flavors. The products should be free of graininess on reconstitution and exhibit no burnt particles.

Dry whole milk is used primarily in confectionary, dairy, and bakery.

DRY BUTTERMILK

Dry buttermilk is the product resulting from the removal of water from liquid buttermilk derived from the churning of butter. It contains not less than 4.5% milk fat and not more than 5% moisture. The protein content of dry buttermilk is not less than 30%. Dry buttermilk is used in dairy foods such as ice cream, and in other foods like bakery, dry mixes, and confectionary.

Dry buttermilk contains higher milk fat than nonfat dry milk. It contains significant level of phospholipids which act as emulsifying agents. Shelf life due to phospholipids is considerably reduced because they are prone to degradation causing fishy odors and flavor defects.

DRY BUTTERMILK PRODUCT

Another form of dry buttermilk is called dry buttermilk product. This designation indicates that it does not meet the specification of protein content of 30% minimum. This product denotes protein content on the label. Except protein content, dry buttermilk product meets all other standards of dry buttermilk.

Dry buttermilk product is the product resulting from the removal of water from liquid buttermilk derived from the churning of butter. It shall contain not less than 4.5% milk fat and not more than 5% moisture. Dry buttermilk product contains less than 30% protein, the label of which should specify the minimum protein content.

Dry milk products are discussed in details in Chapter 14 of this book.

FERMENTED/CULTURED DAIRY PRODUCTS

Approximately, 400 diverse products derived from fermentation of milk are consumed around the world. Fermentation conserves vital nutrients of milk. Simultaneously, it modifies certain milk constituents to enhance their nutritional status, and furnishes to

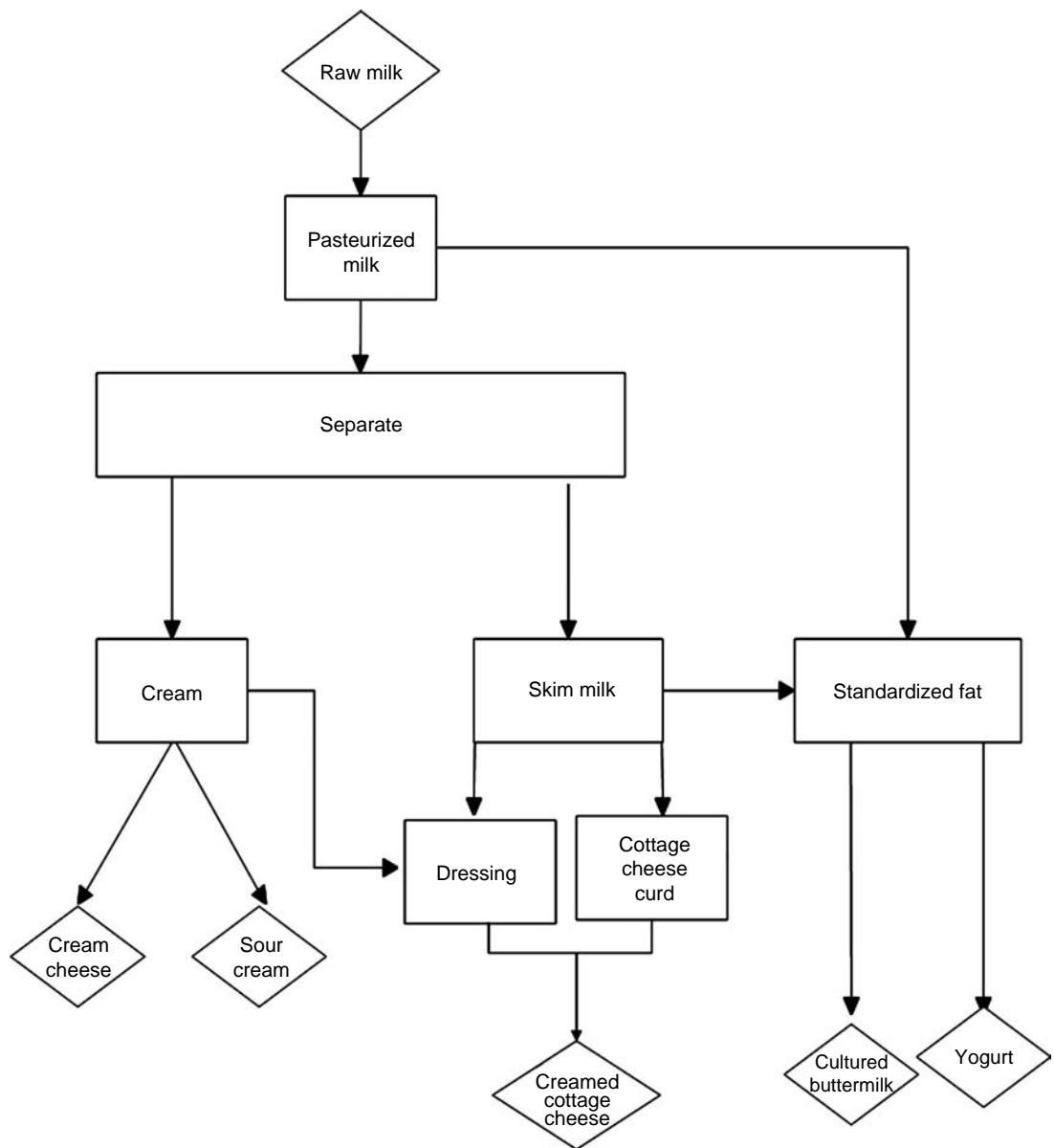


Figure 1.5. An outline of manufacture of cultured dairy products.

the consumer live and active cultures in significant numbers to provide distinct health benefit beyond conventional nutrition. The fermented milk products may be termed as “functional foods.”

Diversity of fermented milks may be ascribed to:

- Use of milk obtained from various domesticated animals,

- Application of diverse microflora
- Addition of sugar, condiments, grains and fruits to create variety of flavors and textures,
- Application of additional preservation methods, for example, freezing, concentrating, and drying.

Figure 1.5 shows an outline for the manufacture of cultured/fermented milks including yogurt, cultured

Table 1.6. Typical Formulation of Certain Types of Yogurt Bases

Composition	Plain Yogurt		Blended Yogurt		
	Low Fat	Full Fat	Full Fat	Low Fat	Nonfat
Milk fat (%)	1.0	3.25–3.50	3.25–3.50	>0.5–<1.2	0.3–0.5
Milk solids-not-fat (%)	14.2	11.0–12.0	10.5–11.0	10.5–12.0	11.0–12.0
Sugar solids (%)	0	0	6.0–10.0	6.0–10.0	6.0–10.0
Stabilizer (%)	0–0.75	0–1.0	0.4–1.6	0.3–1.4	0.3–1.2

Adapted from Chandan and O’Rell (2006b) and Chandan (1997).

buttermilk, sour cream, cream cheese, and cottage cheese.

YOGURT

Yogurt is a semisolid fermented product made from a heat-treated and standardized milk mix by the activity of a symbiotic blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Current trend for using prebiotics and probiotic cultures in the manufacture of fermented milks and yogurt products is in response to consumer expectations for functional or wellness foods. The beneficial effects documented in numerous studies and reviews include prevention of cancer, reduction in diarrhea associated with travel, antibiotic therapy, and rotavirus, improvement of gastrointestinal health, enhancement of immunity of the host, amelioration of lactose tolerance symptoms, protection from infections caused by food-borne microorganisms, control of vaginitis, and vaccine adjuvant effects.

Yogurt is classified into various types. Plain yogurt is the basic style and forms an integral component of fruit-flavored yogurt (Chandan, 2004). It contains no added flavors or sugar.

Table 1.6 shows typical formulation of plain and blended style yogurt.

Figure 1.6 shows a flow sheet diagram for the manufacture of plain yogurt.

Fruit-flavored yogurt may have fruit added on the bottom of plain or sweetened yogurt base or may have fruit blended throughout. Figure 1.7 shows process flow diagram for blended style yogurt.

Other types of yogurt are aerated yogurt, frozen yogurt, and yogurt smoothies. Yogurt products now are supplemented with prebiotics, probiotics, and other functional ingredients. Prebiotics are nondigestible food ingredients that improve the host’s health by selectively stimulating the growth and/or activity of the beneficial bacteria of the colon. Probiotics are live organisms introduced into the gastrointestinal system

of humans to improve the balance or metabolic activity of beneficial organisms. Functional ingredients such as plant sterols, omega fatty acids, antioxidants are ingredients shown by clinical trials to promote health, prevent disease, or help in the treatment of certain disorders.

CULTURED BUTTERMILK

Cultured buttermilk is obtained from pasteurized skim or part skim milk cultured with lactococci and aroma producing bacteria leuconostocs. Table 1.7 shows typical formulation for cultured buttermilk.

The product is bottled in paper/plastic containers. Figure 1.8 illustrates the process flow diagram for cultured buttermilk.

SOUR/CULTURED CREAM

Sour/cultured cream manufactured by culturing pasteurized cream with lactococci and aroma-producing bacteria, leuconostocs has butter-like aroma and flavor. Table 1.7 shows typical formulation for sour cream. Crème Fraîche resembles sour cream, except it contains up to 50% fat as compared to 18% fat in sour cream and has a higher pH of 6.2–6.3. Cultured cream is used in making dips.

Figure 1.9 shows the process for manufacture of sour/cultured cream.

CULTURE-CONTAINING MILKS

Culture-containing milks are seeded but unfermented milks delivering significant doses of probiotic microorganisms. The product is based on pasteurized and chilled low-fat milk to which a concentrate of *Lactobacillus acidophilus* culture has been incorporated to deliver a minimum of one million organisms per milliliter. In this case, the growth of the culture is intentionally avoided to preserve the fresh taste of milk. Accordingly, the product is maintained at

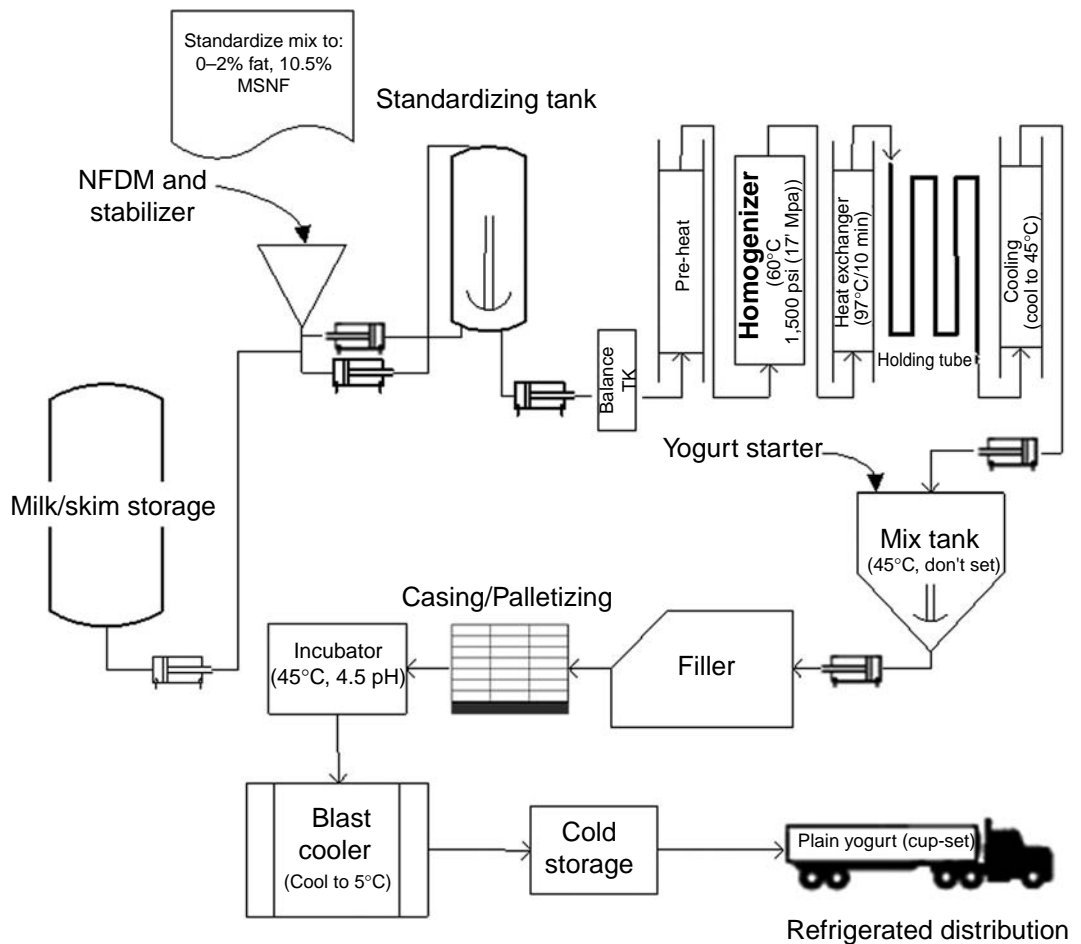


Figure 1.6. Flow sheet diagram for the manufacture of cup-set plain yogurt. From Chandan and O'Rell, 2006b.

refrigeration temperature at all times with a shelf life of 2–3 weeks. Several probiotic organisms like *Bifidobacteria*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Sterptococcus thermophilus* or *Lactobacillus casei* may be included.

SCANDINAVIAN AND EASTERN EUROPEAN FERMENTED MILKS

Scandinavian and Eastern European fermented milks have a distinctive flavor and texture. They are generally characterized by a ropy and viscous body. *Villi*, a fermented milk of Finland is cultured with *Lc. lactis* subsp. *lactis*, *Leuc. mesenteroides* subsp. *cremoris* and a fungus *Geotrichum candidum*. The cream layer traps the fungus giving a typical musty odor to the

product (Mistry, 2001). The fermentation process also elaborates mucopolysaccharides imparting ropiness and viscosity to the product.

Ymer is a Danish product with characteristic high protein (5–6%) and pleasant acidic flavor with butyry notes. The traditional process involves removal of whey by draining curd after fermentation or by inducing separation of whey by heating curd followed by its removal. It is cultured with mesophilic culture consisting of a blend of *Lc. lactis* subsp. *lactis* biovar, *diacetylactis*, and *Leuc. mesenteroides* subsp. *cremoris*.

Skyr is another Scandinavian product. In Iceland, this product is obtained by fermenting skim milk with yogurt culture and a lactose-fermenting yeast. A small amount of rennet may be used to develop

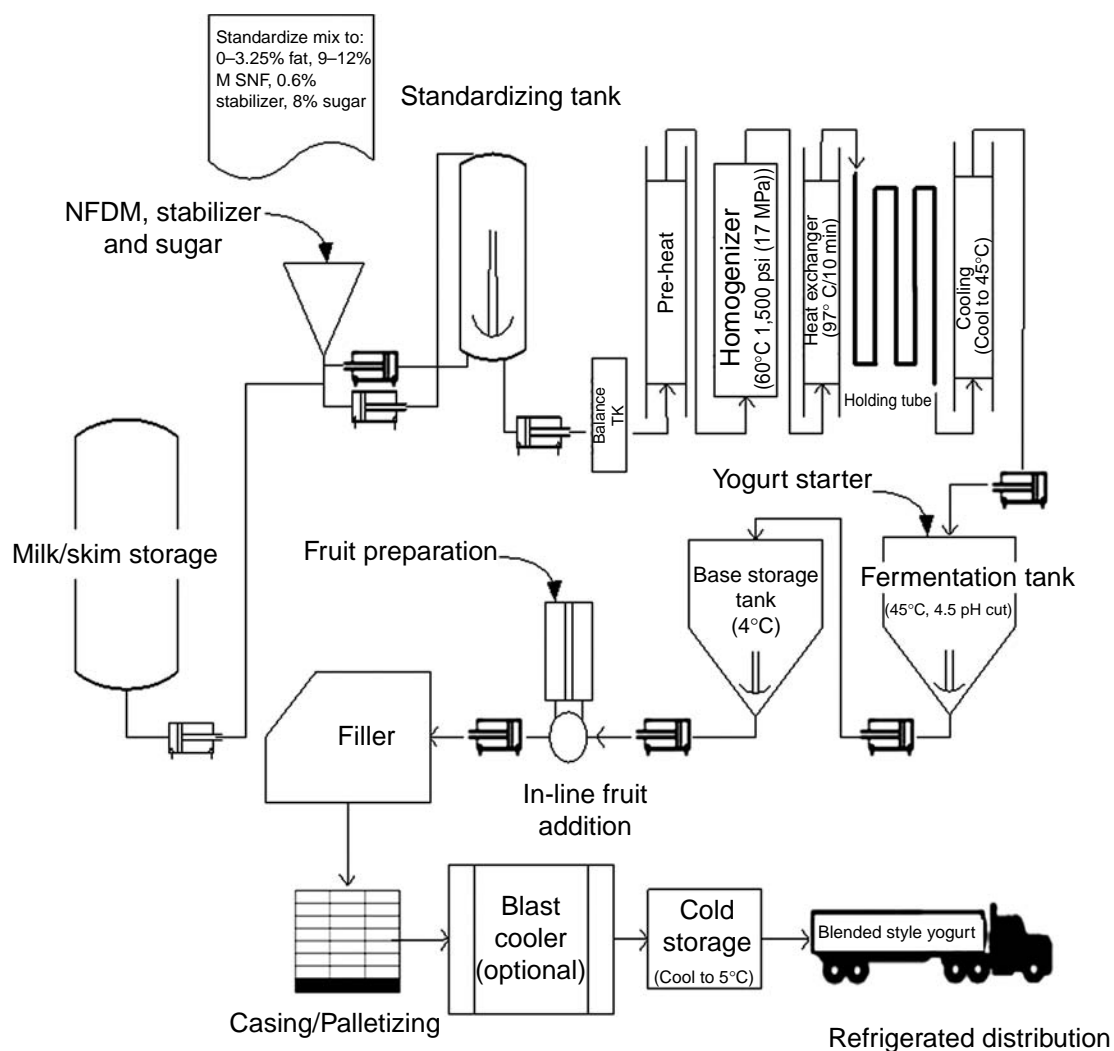


Figure 1.7. Flow sheet diagram for the manufacture of blended style yogurt. From Chandan and O'Rell, 2006b.

Table 1.7. Typical Formulation of Cultured Buttermilk and Cultured (Sour) Cream

Product	Milk Fat (%)	Milk Solids-Not-Fat (%)	Salt (%)	Sodium Citrate (%)
Buttermilk (nonfat)	0.1	10.3	0.18	0.1
Buttermilk (low fat)	1.0–1.2%	10.0	0.18	0.1
Sour cream (regular)	18.5	9.0–10.0	0	0

Adapted from Chandan (1997).

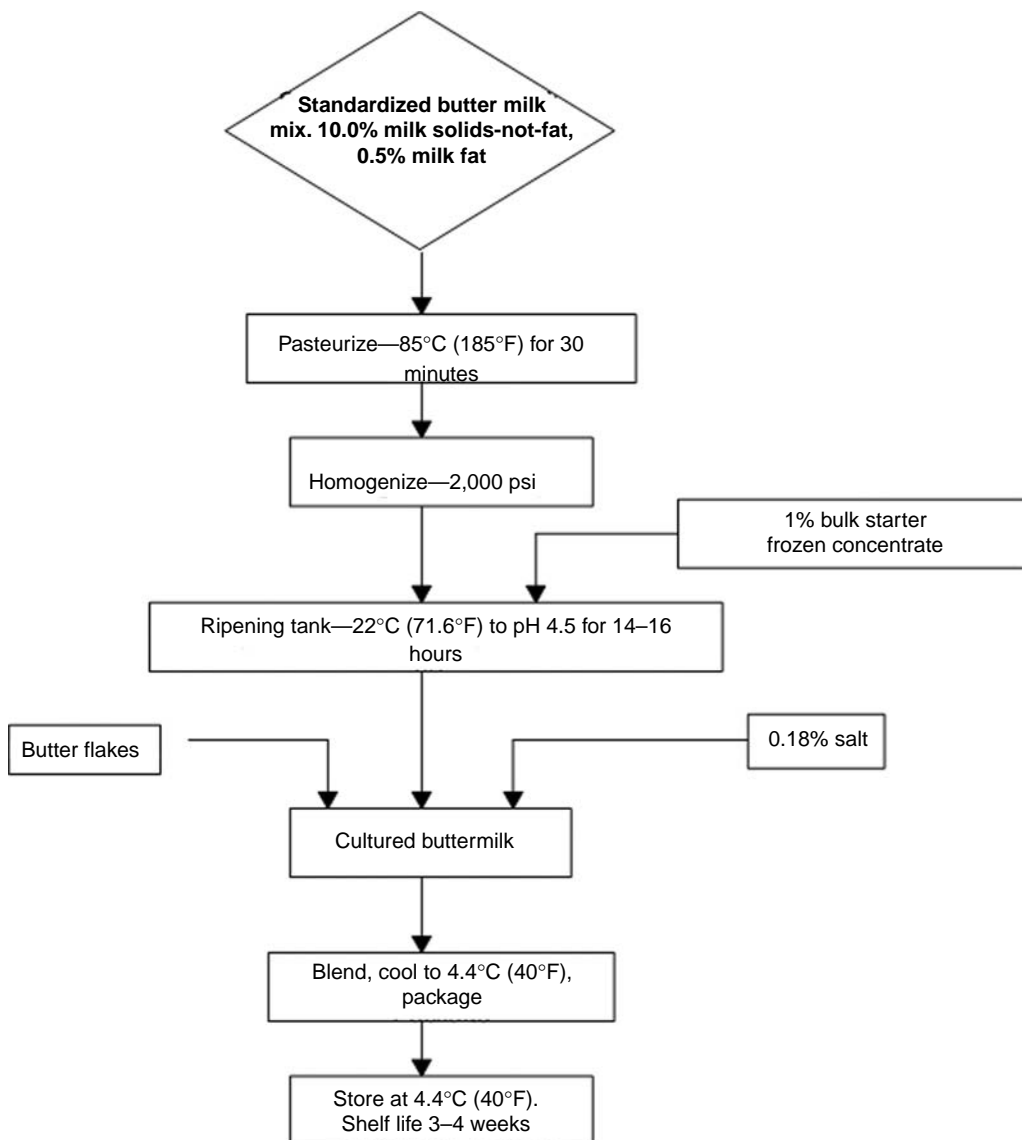


Figure 1.8. An outline of manufacturing cultured buttermilk.

heavier body. Skyr has a flavor profile consisting of lactic acid, acetic acid, diacetyl, acetaldehyde, and ethanol.

Kefi is a popular fermented milk in Russia, Eastern Europe, and certain Asian countries. In addition to lactic fermentation, this product employs yeast fermentation as well. Thus, a perceptible yeast aroma, and alcohol content characterize these products. Also, a fizz is noticed due to production of

carbon dioxide as a result of yeast growth. Kefi utilizes natural fermentation of cow's milk with kefi grains. Kefi grains are a curd-like material, which is filtered off after each use and reused for inoculation of the next batch. Kefi grains contain polysaccharides and milk residue embedded with bacteria *Lb. kefi*, *Lb. kefi ogranum* and species of leuconostocs, lactococci, and lactobacilli. Along with bacteria, the grains contain yeasts including

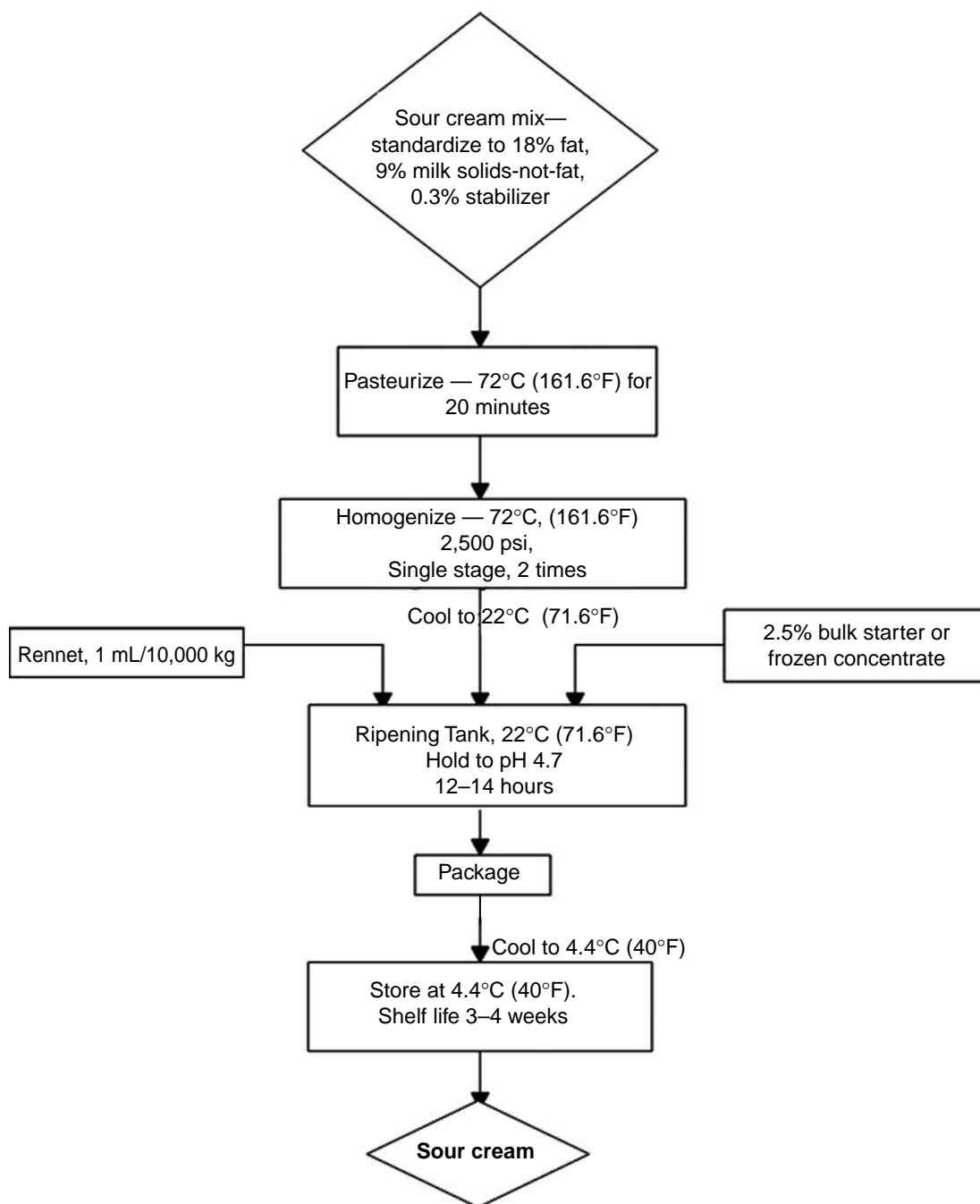


Figure 1.9. A flow diagram for sour cream manufacture.

Saccharomyces kefi, *Candida kefi*, and *Torula species*.

Koumiss is obtained from mare's milk or cow's milk, using a more defined culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, and *Torula* yeasts. This therapeutic product has perceived health benefits.

FERMENTED MILK AND THEIR PRODUCTS OF MIDDLE EAST

Laban rayeb is prepared at home by pouring raw whole milk in clay pots and allow the fat to rise at room temperature. The top cream layer is removed and partially skimmed milk is allowed to undergo spontaneous fermentation. Some variations of the product exist. One is called laban khad, which is fermented in a goat pelt. The other is named Laban zeer, which is distinctly fermented in earthenware pots. The organisms responsible for fermentation are thermophilic lactobacilli in summer season and mesophilic lactococci in winter season (Mistry, 2001).

Kishk is obtained from laban zeer. Wheat grains are soaked, boiled, sun dried, and ground into powder. The blend of wheat and laban zeer is allowed to ferment further for another 24 hours and portioned into small lumps and sun dried. The dried *Kishk* has 8% moisture and 1.85% lactic acid. After proper packaging, its shelf life is of the order of several years. *Kishk* may contain spices. *Labneh* is prepared by concentrating fermented milk after fermentation process is completed. Milk is fermented with yogurt culture and then concentrated using Quarg separator. This product contains 7–10% fat. *Zabady* is an Egyptian product obtained by fermenting milk which has been concentrated by boiling and then fermented with yogurt culture. Further concentration of milk solids is achieved by heating it and separating the whey.

FERMENTED MILK PRODUCTS OF SOUTH ASIA

Dahi is the most common fermented milk in South Asia. Also called curd, dahi is a semisolid product obtained from heat treated and cooled buffalo's milk or a mixture of cow's and buffalo's milk by souring natural or otherwise, by a harmless lactic acid or other bacterial culture. A mixed culture containing *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*, or *Leuconostoc* species, *Lactococcus lactis* subsp. *cremoris* in the ratio of 1:1:1 may be

used. In addition, *Streptococcus thermophilus* may be a component of dahi culture.

For detailed discussion on the manufacture of yogurt and fermented milks, the reader is referred to the book by the same name (Chandan et al., 2006). Chapter 10 in this book contains additional discussion on various fermented milks in the world.

CHEESE AND CHEESE PRODUCTS

Cheese connotes transformation and preservation of vital milk constituents from fluid form to semisolid or solid form. There are at least 400 cheese varieties. The main milk components (proteins, fat, and minerals) are concentrated and protected from rapid deterioration by spoilage microorganisms. Cheese is therefore a concentrated milk food. It provides sound nutrition, variety, convenience of use, portability, food safety, and novelty of flavors and textures to the consumer. Cheese and cheese products are consumed as such or are used as ingredients in entree, side dishes, and ready-to-eat snacks. These products are also designed to be consumed as a spread, as slices in sandwiches and function as a dip or topping on snacks.

NATURAL CHEESE

Natural cheese is made directly from milk (or whey). It is made by coagulating or curdling milk, stirring and heating the curd, draining off the whey, and collecting or pressing the curd. Desirable flavor and texture are obtained in many cheeses by curing process at a specific temperature, humidity, and time period. Typical composition of various cheeses is shown in Table 1.8.

Natural cheeses are classified based on several criteria.

A. Based on Moisture

- Very high moisture cheeses contain 56–80% moisture. These are Cottage, Ricotta, Impastata, Neufchatel, and Cream cheeses.
- High moisture cheeses contain 46–55% moisture and include Mozzarella, Camembert, Brie, Pizza, and Blue cheeses.
- Medium moisture cheeses contain 34–45% moisture and include Edam, Gouda, Brick, Swiss, Cheddar, and Provolone cheeses.
- Low moisture cheeses contain 13–33% moisture and include Romano, Parmesan, Dry ricotta, Mysost, and Gjetost cheeses.

Table 1.8. Typical Composition of Natural and Process Cheeses

Product	Water (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Cottage cheese curd	79.8	0.4	17.3	1.8	0.7
Cottage cheese, Creamed	79.0	4.4	12.5	2.7	1.4
Cream cheese	53.7	34.9	7.5	2.7	1.2
Neufchatel	62.2	23.4	10.0	2.9	1.5
Limburger	48.4	27.2	20.0	0.6	3.8
Camembert	51.8	24.3	19.7	0.5	3.7
Brie	48.4	27.7	20.7	0.5	2.7
Feta	55.2	21.3	14.2	4.1	5.2
Brick	41.1	29.7	23.3	2.8	3.2
Munster	41.8	30.0	23.4	1.1	3.7
Blue	42.4	28.7	21.4	2.2	5.1
Roquefort	39.5	30.6	21.5	2.0	6.4
Gorgonzola	36.0	32.0	26.0	1.0	5.0
Cheddar	36.8	33.1	24.9	1.3	3.9
Colby	38.2	32.1	23.7	2.6	3.4
Swiss	37.2	27.4	28.5	3.4	3.5
Gouda	41.5	27.4	25.0	2.2	3.9
Edam	41.4	27.8	25.0	1.6	4.2
Parmesan	29.2	25.8	35.7	3.2	6.0
Romano	30.9	26.9	31.8	3.6	6.7
Provolone	40.9	26.6	25.6	2.1	4.7
Mozzarella	54.1	21.6	19.4	2.2	2.6
Ricotta	71.7	13.0	11.3	3.0	1.0
Primost	13.8	30.2	10.9	36.6	NA
American process cheese	39.2	31.2	22.1	1.6	5.8
American process cheese food, cold pack	43.1	24.5	19.7	8.3	4.4
American process cheese spread	47.6	21.2	16.4	8.7	6.0
Swiss process cheese	42.3	25.0	24.9	2.1	5.8
Swiss process cheese food	43.7	24.1	21.9	4.5	5.8

Adapted from Nath (1993).

B. Based on Texture and Body

- *Very hard cheeses* (grating) types and ripened by bacteria include Asiago old, Parmesan, Romano, Sapsago, and Spalen.
- *Hard cheeses*, ripened by bacteria and cheese *without eyes* include Cheddar, Granular or Stirred curd, and Caciocavallo. In addition, hard cheeses, ripened by bacteria but cheeses with eyes include Swiss, Emmental, and Gruyere.
- *Semisoft cheeses*, ripened mainly by bacteria include Brick and Muenster. Cheeses ripened by bacteria and surface microorganisms are Limburger, Port du Salut, and Trappist. Cheeses ripened by blue mold in the interior

include Roquefort, Gorgonzola, Blue, Stilton, and Wensleydale.

- *Soft cheeses* may be ripened or unripened. The ripened soft cheeses include Bel Paese, Brie, Camembert, Cooked, Hand, and French Neufchatel. The unripened soft cheeses include Cottage, Pot, Bakers, Cream, Quarg, Tvorog, Neufchatel, Mysost, Primost, and Ricotta.

C. Based on Curing/Ripening and Type of Ripening

- *The unripened cheeses* are made by coagulating milk with acid generated by culturing include cheeses like Cottage, Cream, Neufchatel, Quarg, and Tvorog. Cheeses made

by direct acidification of hot milk include Latin American white cheeses and Paneer (Chandan, 2007c).

- *The ripened cheeses* are made by rennet addition and culturing.

Bacterial ripened cheeses may be ripened by internal bacteria. They include Cheddar, Swiss, Colby, Edam, Gouda, Gruyere, Romano, Provolone, and Parmesan. However, the bacterial ripening may be on the surface of cheese body. Such cheeses are Brick, Trappist, Limburger, Muenster, Bel Paese, Monterey Jack, and Port de Salut.

The mold ripened cheeses may be internal or external ripened. The internally ripened mold cheeses are Blue, Roquefort, Gorgonzola, and Stilton. The surface ripened mild cheeses include Camembert and Brie cheeses.

Figure 1.10 shows an outline for manufacture of Cream cheese.

Figure 1.11 illustrates basic steps in the manufacture of Cheddar and Mozzarella cheeses.

PASTEURIZED PROCESS CHEESE

Pasteurized process cheese is the food prepared by comminuting and mixing, with the aid of heat, one or more cheese of the same or two or more varieties (except cream cheese, Neufchatel cheese, cottage cheese, creamed cottage cheese, cook cheese, hard grating cheese, semisoft part-skim cheese, part-skim spice cheese, and skim milk cheese for manufacturing) with an emulsifying agent into a plastic homogeneous mass. Heating is at not less than 65.5°C (150°F) and for not less than 30 seconds. The moisture content is required not to exceed 1% more than constituent natural cheeses, but cannot exceed 43%.

Process cheese is a pasteurized blend of American cheeses of different ages. It comes in different flavors. American process cheese has mild Cheddar flavor. Sharp American has sharp or aged Cheddar flavor. American Swiss has mild Swiss flavor. Consistency of process cheese is relatively semifirm (creamy, and smooth as compared to natural cheese counterparts). Functionalities available are sliceability, extra-melt (melting easily on heating, does not thicken and can withstand high temperature hold for long periods), and slow melt (maintains shape at high temperature). It may be flavored with seasonings. Moisture content is 40% maximum and fat in dry matter is 50% minimum. Figure 1.12 illustrates the main steps involved in the production of process cheese, loaf, and slices.

PASTEURIZED PROCESS CHEESE FOOD

Pasteurized process cheese food is similar to pasteurized process cheese, except it must contain moisture not exceeding 44%, and fat content is not less than 23%. It contains optional dairy ingredients: cream, milk, skim milk, buttermilk, cheese whey solids, anhydrous milk fat, and skim milk cheese for manufacturing. The pH is adjusted to not below 5.0 with vinegar, lactic acid, citric acid, phosphoric acid, and acetic acid. It cannot contain more than 3% emulsifying agents, and 0.2% sorbic acid. It is obtained by blending American cheeses of different ages with nonfat dry milk and whey and other permissible ingredients, followed by pasteurization. It melts quickly to give a smooth liquid. Cold product can be sliced easily. Major uses include entrees, Au Gratin potatoes, sandwiches, and Mexican dishes. It may be flavored with seasonings, smoke, pimento, jalapeno, salami, pepperoni, and so forth. Moisture content is 44% maximum and fat in dry matter is 41% minimum.

PASTEURIZED PROCESS CHEESE SPREAD

Pasteurized process cheese spread contains even higher moisture and lower fat than process cheese food. It is more spreadable than cheese food. It may contain meat, vegetables, pimento, pineapple, or may be flavored with blue cheese, onion, and so forth. Its uses include snacks, deviled eggs, noodle casserole, meat balls, hot vegetables, sandwiches, sauces, and dressings.

It is similar to process cheese food, but is spreadable at 21°C (69.8°F). It has a moisture content of 44–60% and fat content is not less than 20%. It may contain optional dairy ingredients, emulsifying agents, and gums (<0.5%). Acids may be added to get pH to not less than 4.0. Sweetening agents may be used (sugar, dextrose, corn sugars). Sorbic acid (<0.2%) may be used as a preservative.

COLD PACK CHEESE (CLUB CHEESE)

Cold pack cheese (club cheese) is a cold blend of American cheese or Swiss cheese and may be smoke flavored. It spreads easily and is used as an appetizer, snack, or dessert. The product involves blending without heating various cheeses. Only cheese from pasteurized milk is used. Its moisture content is the same as individual cheese; the fat content in dry matter is not less than 47% in most cheese except Swiss

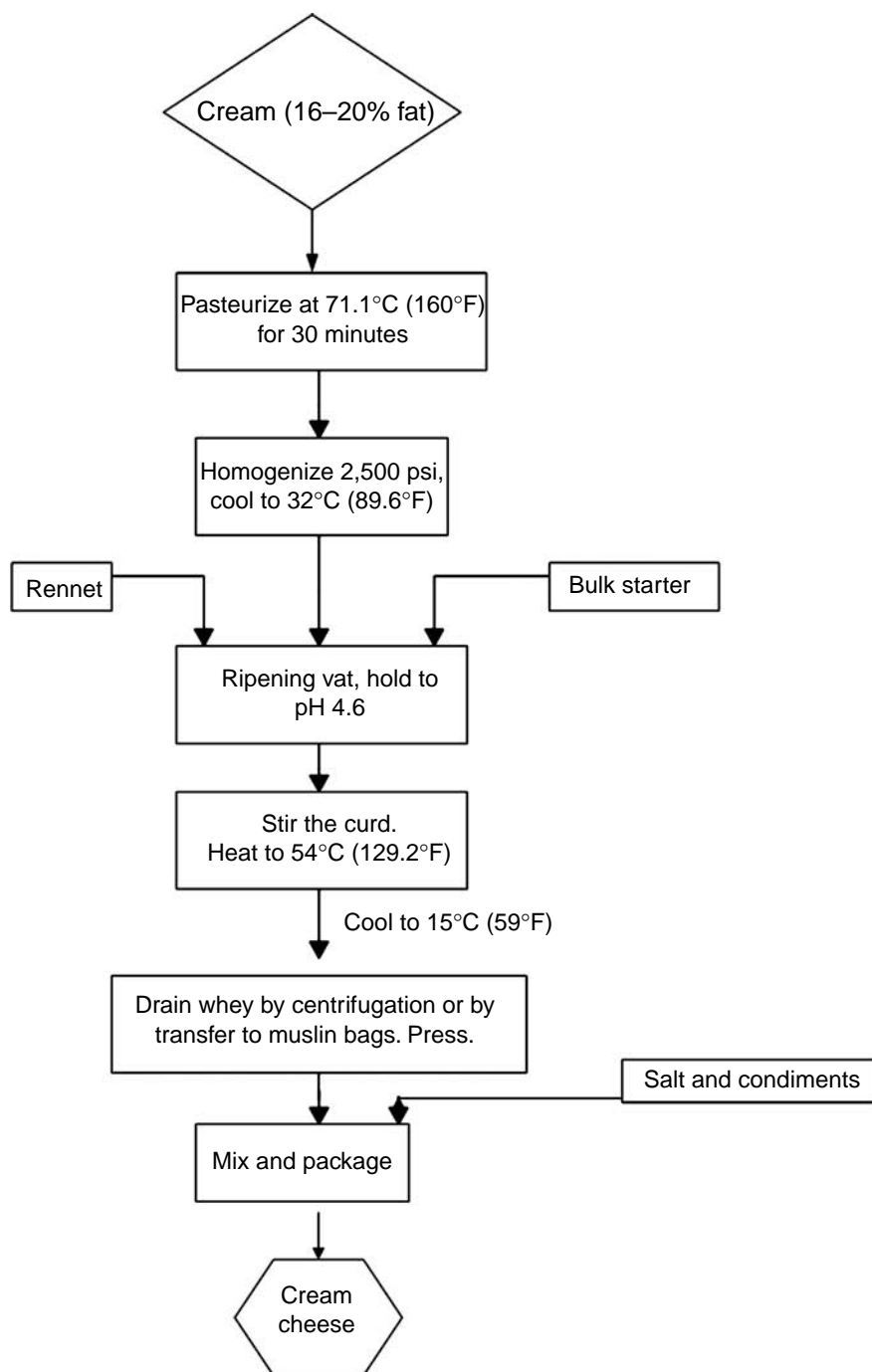


Figure 1.10. An outline for manufacturing cream cheese.

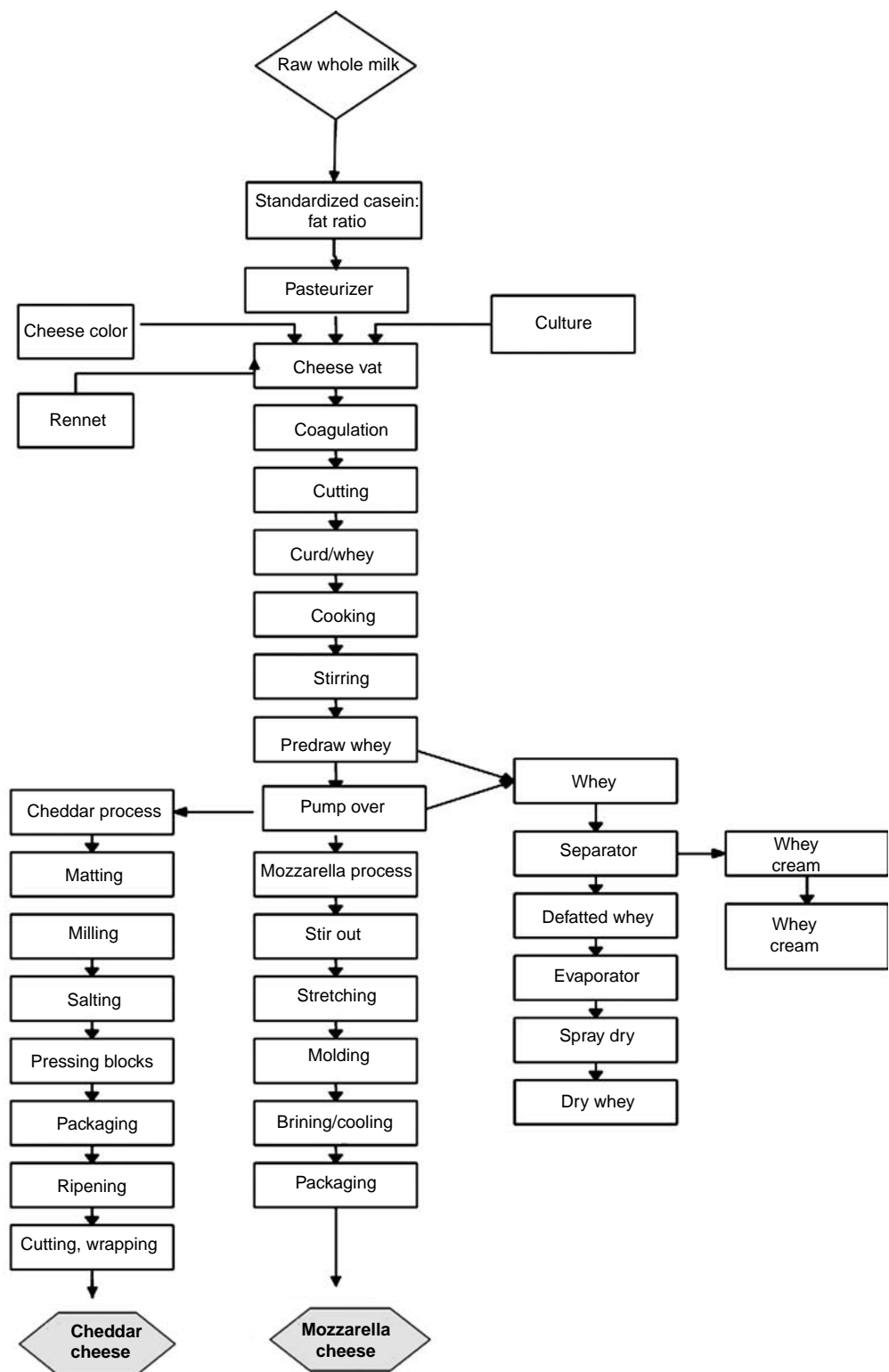


Figure 1.11. An outline of natural cheese manufacture.

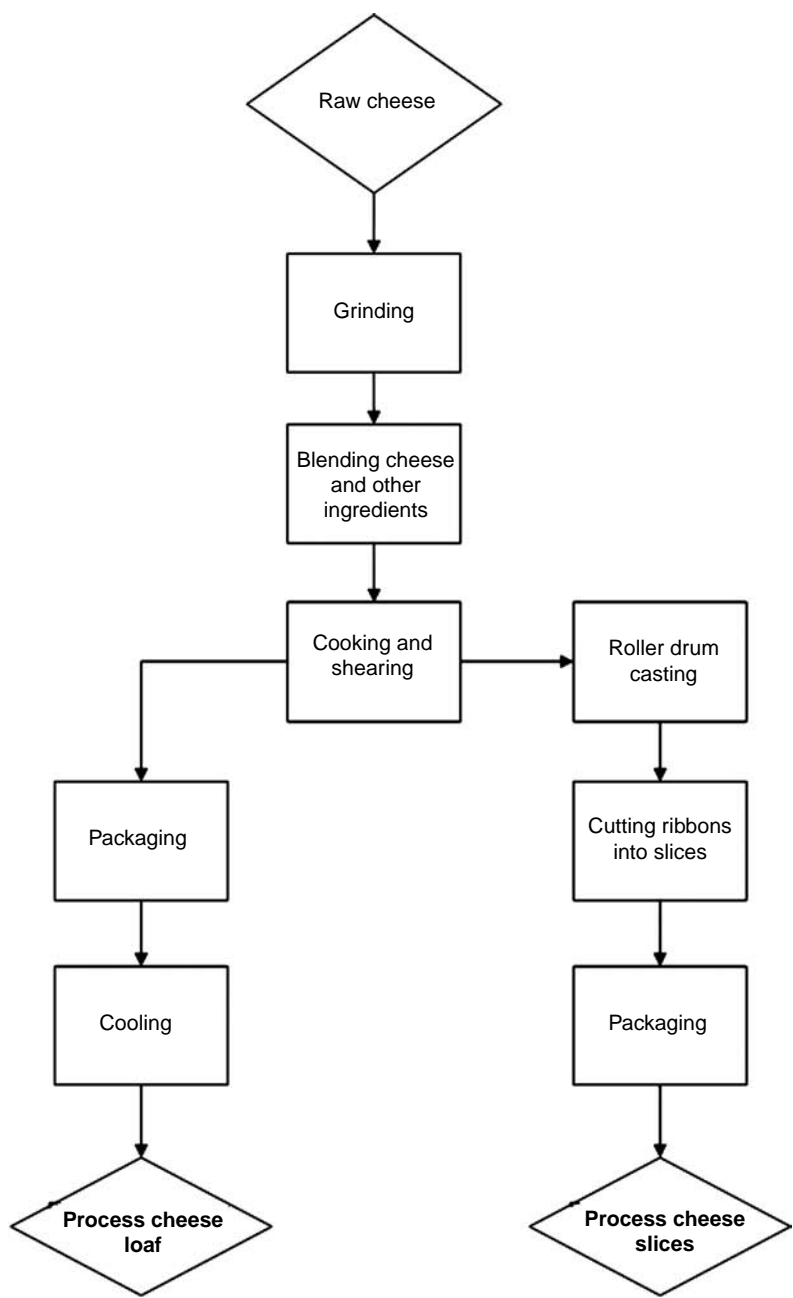


Figure 1.12. A manufacturing outline of processed cheese products.

(not less than 43%) and Gruyere (not less than 45%). Cold pack cheese may contain acids to standardize pH to not below 4.5. Sorbic acid (<0.3%) can be used as preservative.

COLD PACK CHEESE FOOD

Cold pack cheese food is prepared by comminuting and mixing (without heating) cheeses and other ingredients like cream, milk, skim, buttermilk, whey

solids, and anhydrous milk fat. Acids may be added to pH not <4.5. Sweetening agents (sugar, corn solids) may also be used. Sorbic acid (0.3%) may be used as a preservative. Guar gum or xanthan gum may be used (0.5%). Moisture content cannot exceed 44% and fat content is not less than 23%. It may be smoke flavored. It is more spreadable than cold pack cheese. Its uses are the same as for cold pack cheese.

CHEESE POWDERS

Spray-dried cheese powders are widely used as seasonings and flavorings in grain-based snacks. They are produced by macerating cheese, dispersing in water at 35–40% solids concentration, adding emulsifying salts, homogenizing, and spray drying. Foam spray drying is considered to give a superior flavored product with larger particle size. In addition to cheese, dry milk, whey, vegetable oils, salt, enzyme-modified cheese concentrate, color, and seasonings may also be incorporated in the ingredient.

Cheese powders can be packed in nitrogen atmosphere to give a longer storage life. Hard Italian cheese (namely, Parmesan) is dried after grating in tray or belt dryers in which dry hot air is circulated to reduce moisture to less than 6%. After cooling, the cheese is ground and packaged.

ENZYME-MODIFIED CHEESES

Enzyme-modified cheeses (EMC) are cheese flavor concentrates obtained by treating raw cheese curd with specific lipases and proteases along with fermentation with a cheese culture. It takes 1–3 days to develop flavor concentration of 10- to 20-fold as compared to ripened cheeses. Cheese paste is then heat treated to stop the biochemical reaction, and cooled. The EMC may be purchased as a paste or it may be blended with whey and dried as a spray-dried powder. It offers significant savings as a substitute of aged cheese in cheese flavored crackers and other bakery items. Also, it is an economical ingredient in process cheese manufacture.

CHEESE SAUCES

They are aseptically processed slurries and canned for convenient use as a dip or as a sauce on nachos, potatoes, and so forth. Typically, ingredients used are Cheddar cheese, skim milk, whey, buttermilk, vegetable oil, starch, sodium phosphate, salt, caseinate, citrate, color, lactic acid, stabilizers, emulsifiers, and seasonings.

For more detailed discussion on cheese, the reader is referred to Chapter 12 of this book.

WHEY PRODUCTS

Whey, the greenish-yellow liquid produced from the manufacture of cheese, contains about half of the solids of whole milk. Its composition depends largely on the variety of cheese being made. These solids are valuable additions to the functional properties of various foods, as well as a source of valuable nutrients. The techniques of concentration, drying, and reverse osmosis recover all of the whey solids. Crystallization, ion exchange, and membrane systems such as ultrafiltration and electrodialysis effect fractionating whey into concentrates of protein, minerals, and lactose.

Figure 1.13 shows an outline for the manufacture of whey products and milk protein concentrate.

The proximate composition of dry whey, whey products, caseinates, and milk protein concentrates is shown in Table 1.9.

DRY SWEET WHEY

Dry sweet whey is produced by drying of defatted fresh whey obtained from the manufacture of Cheddar, Swiss, and other cheeses. It contains all the constituents except water in the same relative proportion as in liquid whey.

Dry acid whey is similar to dry sweet whey but is produced by drying of fresh whey obtained from Cottage and Ricotta cheese manufacture.

Spray drying of condensed whey converts sweet whey into a stable, nonhygroscopic, and noncaking product. In this process, high solids whey concentrate is spray dried to a free moisture content of 12–14%, causing lactose to take on a molecule of water and become crystallized. This causes whey solids to convert from a sticky, syrupy-like material into a damp powder with good flow characteristics. For drying acid cottage cheese whey, a commercial dryer combines spray drying, with through-flow continuous bed drying. The concentrate is spray dried in the hot air chamber to 12–15% moisture. The particles fall to a continuous, porous, stainless-steel belt where lactose undergoes rapid crystallization. Crystallization of lactose before final drying is necessary for drying acid whey. A belt conveys the product to another chamber where the whey is further dried by dehumidified air that moves through the porous bed.

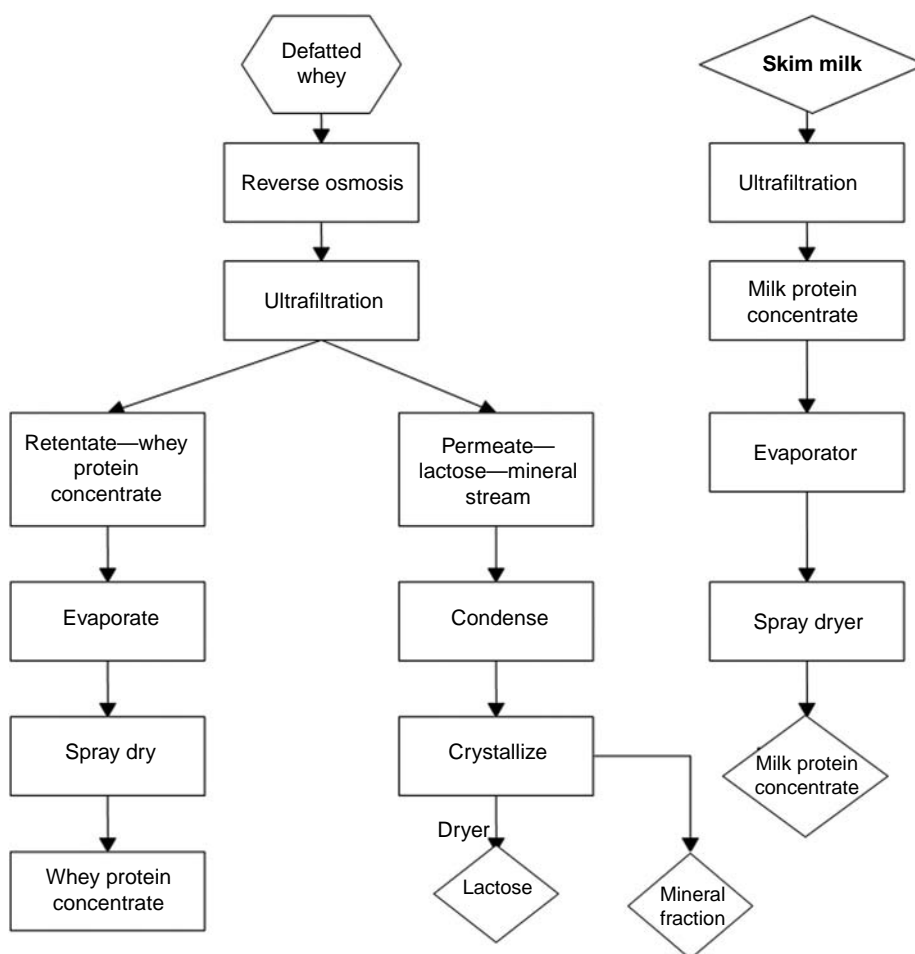


Figure 1.13. A flow sheet diagram for whey processing.

Dry sweet whey is widely used in bakery products, dry mixes, process cheese foods and spreads, frozen desserts, sauces, meat emulsions, confections, soups, gravies, snack foods, and beverages. Dry acid whey has an additional functional attribute of providing acid flavor in certain foods and it imparts desirable textural properties to bakery items.

FRACTIONATED WHEY PRODUCTS

Membrane technology is used for partial concentration (reverse osmosis), fractionation of solutes (lactose, minerals) from macromolecules like proteins, fat globules, colloidal particles (ultrafiltration) and demineralization (ion exchange, electrodialysis)

of whey, its fractions, and milk. These processes produce highly functional ingredients and are commonly used in whey concentration and fractionation. The two are pressure-activated processes that separate components on the basis of molecular size and shape. Reverse osmosis is the process in which virtually all species except water are rejected by the membrane. The osmotic pressure of the feed stream in such a system often will be quite high. Consequently, to achieve adequate water flow rates through the membrane, such systems often use hydrostatic operating pressures of 5883.6 kg/cm² (600 psi) or greater. Ultrafiltration refers to the process in which the membrane is permeable to relatively low molecular weight solutes and solvent (permeate), but is

Table 1.9. Proximate Composition of Dry Whey and Other Dairy Products

Product	Water (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Dry sweet whey	4.5	1.1	12.9	73.5	8.0
Reduced lactose whey	4.0	2.5	22.0	55.0	16.5
Demineralized whey	4.0	2.2	13.0	76.8	4.0
Dry acid whey	4.3	1.0	12.3	71.3	11.1
Whey protein concentrate (34% protein)	3.5	4.0	34.5	51.0	7.0
Whey protein concentrate (50% protein)	3.5	4.0	50.5	36.0	6.0
Whey protein concentrate (80% protein)	3.5	6.0	80.5	5.0	5.0
Whey protein isolate	3.5	0.5	93.0	1.0	2.0
Acid casein	9.0	1.0	88.0	0.1	1.9
Rennet casein	11.0	1.0	85.0	0.1	2.9
Calcium caseinate	3.5	1.0	91.0	0.1	4.4
Sodium caseinate	3.0	1.5	90.9	0.1	4.5
Milk protein concentrate	4.0	3.0	65.0	22.0	6.0
Food grade lactose	0.5	0.1	0.1	99.1	0.2
Dairy minerals concentrate	10.0	1.0	8.0	1.0	80.0

Adapted from Chandan (1997), Sodini and Tong (2006), and Chandan and O'Rell (2006a).

impermeable to higher molecular weight materials (retentate). The permeability and selectivity characteristics of these membranes can be controlled during the fabrication process so that they will retain only molecules above a certain molecular weight. Thus, ultrafiltration is essentially a fractionating process, while reverse osmosis is effectively a concentrating process.

One advantage of ultrafiltration over other processes is that by varying the amounts of permeate removed, a wide variety of protein concentrates, ranging up to 60% protein, can be obtained. Higher levels can be obtained by simultaneously adding fresh water and further concentrating by ultrafiltration. The permeate is used for manufacture of milk sugar, lactose, by condensing and crystallization. Lactose crystals are harvested and dried in a tumble dryer.

Reduced Lactose Whey

Reduced lactose whey is produced from whey by partial crystallizing out lactose and recovery of mother liquor by centrifugation. Lactose content of the dry product is 60% or less.

Reduced Minerals Whey

Reduced minerals whey is produced from whey by selective removal of a portion of minerals. Ash

content of the dry product is 7% or less. Demineralization processes have helped in the development in an array of whey products. Excessive mineral content makes whey distasteful, and they can have an adverse effect on the physical properties of some foods. The two most widely used demineralization processes for whey are ion exchange and electrodialysis.

In the ion-exchange process, whey is passed through two containers which are filled with special synthetic resins which have the ability to exchange ions. In the first container, the special synthetic resins exchange hydrogen ions for cations in the whey. Here, the positive ions of the salt are captured and acid is formed by the release of hydrogen ions. The whey is then passed over the anion exchanger where hydroxyl ions are exchanged for negative ions of the salt, and water is formed. When the mobile ions of the resins are completely replaced by other ions, the resin must be regenerated for further use. This is done by passing an acid (hydrochloric) solution through the cationic exchanger, and a basic solution (sodium chloride) through the anionic exchanger.

Electrodialysis, a combination of electrolysis and dialysis, is the separation of electrolytes, under the influence of an electric potential through semipermeable membranes. The driving force is an electric field between the anode (positively charged) and the cathode (negatively charged). Between the anode and the cathode, a number of ion-selective membranes are

placed which are permeable only to anions or cations. Every other membrane has a positive charge repelling positive ions and allowing negative ions to pass, and in between there is a negatively charged membrane doing just the opposite.

In principle, whey is pumped through every second space between two membranes, and a solution of sodium chloride (cleaning solution) is pumped through the compartments between the whey streams. The ions move from the whey stream into the cleaning solution where they are retained, because they cannot move any further. The cleaning solution contains minerals, acid, some lactose, and small nitrogenous molecules. The membranes are cleaned chemically. Protein molecules remain in the fluid while the minerals are removed. The process results in a protein concentrate.

Lactose

Lactose is crystallized from condensed whey or from permeate (50–60%, solids) obtained by ultrafiltration fractionation of milk or whey. The supersaturated solution is cooled under specific conditions to crystallize lactose. Lactose crystals are harvested and washed to remove the mother liquor and dried. Crude lactose obtained this way contains approximately 98% lactose. Edible and USP grades are produced from crude lactose by protein precipitation, decolorization with activated carbon and subsequent demineralization. Lactose is further refined by recrystallization, followed by spray drying.

Whey Protein Concentrates and Isolates

Whey protein concentrates are products derived from whey by removal of minerals and lactose. The process of protein concentration utilizes ultrafiltration, electrodialysis, and ion-exchange technologies. On dry basis, the protein concentrate contains a minimum of 25% protein. Whey protein isolate contains at least 92% protein.

Whey protein concentrate of 34% protein is commonly used as a stabilizer in yogurt, bakery mixes, dietetic foods, infant foods, and confections. Its water binding, fat-like mouth-feel, and gelation property is particularly useful in these products. Wheat protein concentrate of 50% or 80% protein offers distinct functional attributes. It is especially suited for use in nutritional drinks, soups, bakery, meat, dietary foods, and protein fortified beverages. It gives clear suspensions over a wide pH range and has a bland flavor.

Some applications require undenatured ingredients to maximize water-binding capacity during food processing. It is also available in gel-forming version.

Chapter 15 of this book gives details of various aspects of whey and whey products.

OTHER DRY MILK PRODUCTS

In this category are products such as casein, caseinates, and milk protein concentrates.

Casein and Caseinates

Casein represents products obtained from pasteurized skim milk by precipitation of casein fraction of milk protein using an acid, followed by drying. This gives acid casein. Casein derived from the action of rennet (chymosin) is called rennet casein. Micellar casein is also commercially available. All of them have distinctive functional characteristics.

Caseinates are derived from casein by treatment with a suitable alkali. Casein is basically insoluble in water, whereas caseinates are easily dispersible. Acid casein is produced by precipitation of skim milk with hydrochloric acid, sulfuric acid, acetic acid, or lactic acid at pH 4.6. Acid casein is neutralized to pH 6.7 with sodium hydroxide for the production of sodium caseinate. Similarly, potassium hydroxide and calcium hydroxide yield potassium and calcium caseinates, respectively.

Milk Protein Concentrate

Milk protein concentrate is obtained by ultrafiltration of skim milk and subsequent spray drying. Protein content varies according to the application in dairy products. An outline for the manufacture of milk protein concentrate is also shown in Figure 1.13.

REFRIGERATED DAIRY DESSERTS/SNACKS

This category includes puddings, custards, cheese cake, and other products sold in refrigerated form. They are discussed in Chapter 17.

ICE CREAM AND FROZEN DESSERTS

Ice cream is a food produced by freezing, while stirring, a pasteurized mix consisting of ingredients defined by Food and Drug Regulations. Ice cream is

a frozen blend of air, water, milk fat, milk solids-not-fat, sweeteners, stabilizers, emulsifiers, flavorings such as fruits, nuts and chocolate chips, and coloring materials. Regular ice cream must contain at least 10% fat before the addition of bulky flavorings and must weigh at least 4.5 pounds/gallon. There are other products in frozen desserts category. Frozen custard or French ice cream must contain at least 10% milk fat and 1.4% egg yolk solids. Sherbet contains 1–2% milk fat, higher sweetener level than ice cream and must weigh not less than 6 pounds/gallon. Sherbet is commonly flavored with fruit and fruit juices. Sorbet and water ice resemble sherbet but contains no dairy ingredients. Frozen yogurt is made of milk/skim milk, nonfat dry milk/condensed milk, cream, yogurt, sweeteners and corn syrup solids, stabilizer emulsifiers and flavorings.

All frozen dessert mixes are formulated, processed, and extruded through ice cream freezers to deliver desirable consumer attributes of flavor, texture, and shelf life.

Frozen desserts may be labeled as low fat or non-fat depending on their fat contribution per serving of 1/2 cup (65–70 g). Products containing no more than 3 g fat/serving are classified as low fat. Nonfat products contribute less than 0.5 g fat/serving. Reduced fat products provide less than 25% lower fat than reference product.

Dairy ingredients constitute 50–55% of the total solids of ice cream and related frozen desserts. Choice of dairy ingredients and formulation of an ice cream mix are determined by the regulatory standards, desired quality of the frozen dessert, marketing

strategy, consumer demand, relative prices, and availability of the ingredients in a given locality.

The milk products constitute the most important ingredients because they furnish the basic ingredients for a good quality ice cream. Variables related to dairy ingredients exert a profound influence on flavor, body, and texture of the frozen product. The nature and intensity of overall ice cream flavor is a function of the flavor quality of individual constituents and subsequent processing treatment accorded the ice cream mix. Flavor defects in the ingredients cannot be alleviated during ice cream making. Actually, flavor problems could be compounded as a consequence of negligent processing procedures.

The body or consistency of ice cream is related to mechanical strength of the mix and its resistance to melting. Heat shock resistance is dependent on the nature and concentration of stabilizer–emulsifier system used. The texture of ice cream depends upon the size, shape, number, and arrangement of air cells, fat globules, ice crystals, and ratio of frozen and liquid water in ice cream.

An important variable related to foam formation as a result of aeration of ice cream mix during freezing process is called overrun. Overrun is the volume of ice cream obtained over and above the volume of mix used. For instance, if the volume of ice cream is double of the ice cream mix, the overrun is 100%.

Mellorine is similar to ice cream except that it contains no milk fat which is substituted with vegetable fat in the formulation.

Table 1.10 shows typical formulation for ice cream and frozen dessert mixes.

Table 1.10. Typical Formulation of Ice Cream Mix and Sherbet Mix

Component	Economy Ice Cream	Regular Ice Cream	Premium Ice Cream	Super Premium Ice Cream	Low-Fat Ice Cream	Sherbet
Milk fat (%)	10.1	10–12	12	16–18	3	1.5
Nonfat milk solids (%)	7.5	7.5–10	9	9–10.5	13	3.5
Whey solids (%)	2.5	0–2.5	0–2	0	0	
Sucrose (%)	0	6–12	7–8	15.5	9	23
55 DE high fructose corn syrup (%)	7.6–9.0	0–6	4–5	0	0	0
36 DE corn syrup solids (%)	9–11.4	6	9	0	9	7
Stabilizer (%)	0.3	0.25	0.35	0–0.12	0.6	0.3
Emulsifier (%)	0.1	0.1	0.25	0.1	0.2	0.1
Total solids (%)	37–40	38–40	40.50	40–42	34.8	35.4

DE, dextrose equivalent.

Adapted from Kilara and Chandan (2007).

For the manufacture of ice cream and frozen desserts, the liquid ingredients are blended in a processing vat. The dry ingredients are added to the liquid blend through a powder funnel or high shear mixing equipment. The mix is pasteurized at 79.4°C (175°F) for 25 seconds and homogenized at 57.2–62.8°C (135–145°F) at 2,000 psi, first stage and 500 psi, second stage. The mix is then cooled to less than 4.4°C (40°F) and extruded through an ice cream freezer at –6°C (–21.1°F). It is packaged and hardened at –30 to –35°C (–22 to –31°F) (Kilara and Chandan, 2007; Schmidt, 2004).

SOFT FROZEN DAIRY PRODUCTS

These products constitute soft-serve ice cream which is served immediately from the ice cream freezer. Milk shakes also belong to this category. These products are generally lower in fat than their hard-pack counterparts. Fat substitutes based on starch, pectin, and whey proteins along with gums, cellulose gel, microcrystalline cellulose, maltodextrins, sodium caseinate, and so forth, provide body and texture to the product. Serum solids content varies from 10 to 16% and the total solids vary from 30 to 35%. In comparison with hard ice cream, soft frozen desserts contain higher serum solids and lower sweetener level. The draw temperature is also lower –6.7 to –7.8°C (18–20°F) for soft-serve products.

Chapter 16 describes in detail the manufacture of ice cream and frozen desserts.

NUTRIENT PROFILES OF DAIRY FOODS

Milk and milk products are composed of unique nutrients providing complete nutritional needs of the neonate. Most nutrition experts recognize milk and milk products as vital constituents of a balanced diet for humans of all ages. Chapter 18 of this book is dedicated to the nutrition and health aspects associated with milk and dairy foods.

Tables 1.11–1.13 show nutrient profile relative to fluid milk and cream products, yogurt, buttermilk, sour cream, butter, and certain cheeses.

The contribution of crucial nutrients is corroborated by the data shown in the Tables 1.11–1.13. Among other vital functions, they provide significant quantities of calcium, protein, vitamins, and essential fatty acids. Furthermore, milk and dairy products are now gaining recognition as discrete functional foods or wellness foods in human diet.

Milk and dairy ingredients are now recognized for their role beyond conventional nutrition (functional attributes) in human health maintenance (Chandan, 2007b; Chandan and Shah, 2007).

QUALITY ASSURANCE

A successful dairy operation must manufacture safe and wholesome milk and dairy products. It is imperative to maximize customer satisfaction, minimize product loss, and comply with sanitary codes. The quality of the dairy product equates survival and growth of the business. Factors such as plant conditions, manufacturing practices, housekeeping, sanitary standards, personal hygiene, and work habits of employees and visitors assume critical importance in plant control of quality, product safety, personal safety, and financial integrity.

GOOD MANUFACTURING PRACTICES

The plant must conform to good manufacturing practices as defined by the Food and Drug Administration. This regulation details various standards for floors, walls, doors and windows, lighting, ventilation, water supply, plant cleanliness, disposal of wastes, and sanitary personnel practices.

HAZARD CONTROL AND CRITICAL CONTROL POINTS

HACCP is useful in safe manufacture of a food product. Defining critical control points (CCPs) helps to eliminate or control hazardous microorganisms or their toxins at any point during the entire production sequence. HACCP information is available in the USFDA publication “Pasteurized Milk Ordinance, 2003.” The reader is referred to the website: <http://www.cfsan.fda.gov/~ear/pmo03jk.html#appk>. Excerpts from the FDA document are given below to introduce the concept of HACCP to the readers.

HACCP is a management tool that provides a structured and scientific approach to the control of identified hazards. It is currently a voluntary program.

The HACCP concept enables those operating under and regulating under a HACCP plan to move to a preventive approach, whereby potential hazards are identified and controlled in the manufacturing environment that is prevention of product failure.

The following are the seven HACCP principles to be included in a HACCP Plan:

Table 1.11. Typical Nutrient Profile of Fluid Milk and Cream Products

Nutrient	Nonfat/Fat Free/Skim Milk	Low-Fat Milk (1% Fat)	Reduced Fat Milk (2%)	Whole Milk (3.3% Fat)	Half and Half (10.5% Fat)	Light/ Coffee Cream (18% Fat)	Whipping Cream, Heavy (36% Fat)
Weight of a serving	1 cup (245 g)	1 cup (244 g)	1 cup (244 g)	1 cup (244 g)	1 tbsp (15 g)	1 tbsp (15 g)	1 tbsp (15 g)
Moisture (%)	91	90	89	88	81	74	58
Calories (kcal)	86	102	121	150	20	29	52
Protein (g)	8	8	8	8	Tr	Tr	Tr
Total fat (g)	Tr	3	5	8	2	3	6
Saturated fatty acids (g)	0.3	1.6	2.9	5.1	1.1	1.8	3.5
Monosaturated fatty acids (g)	0.1	0.7	1.4	2.4	0.5	0.8	1.6
Polyunsaturated fatty acid (g)	Tr	0.1	0.2	0.3	0.1	0.1	0.2
Cholesterol (mg)	4	10	18	33	6	10	21
Carbohydrate (g)	12	12	12	11	1	1	Tr
Total dietary fiber (g)	0	0	0	0	0	0	0
Calcium (mg)	302	300	297	291	16	14	10
Iron (mg)	0.1	0.1	0.1	0.1	Tr	Tr	Tr
Potassium (g)	406	381	377	370	19	18	11
Sodium (mg)	126	123	122	120	6	6	6
Vitamin A (IU)	500	500	500	307	65	95	221
Thiamin (mg)	0.09	0.10	0.10	0.09	0.01	Tr	Tr
Riboflavin (mg)	0.34	0.41	0.40	0.40	0.02	0.02	0.02
Niacin (mg)	0.20	0.32	0.20	0.2	Tr	Tr	Tr
Ascorbic acid (mg)	2	2	2	2	Tr	Tr	Tr

Tr, Trace.

Source: United States Department of Agriculture (2002).

Table 1.12. Typical Nutritional Profile of Yogurt

Nutrient Per 8 oz Serving (227 g)	Plain, Nonfat	Plain, Low Fat	Plain, Whole Milk	Fruit-Flavored, Nonfat	Fruit-Flavored, Low Fat	Light, Vanilla Nonfat
Moisture (%)	85	85	88	75	74	87
Calories (kcal)	127	144	139	213	231	98
Protein (g)	13	12	8	10	10	9
Total fat (g)	0.5	4	7	Tr	2	Tr
Saturated fatty acids (g)	0.3	2.3	4.8	0.3	1.6	0.3
Monosaturated fatty acids (g)	0.1	1.0	2.0	0.1	0.7	0.1
Polyunsaturated fatty acid (g)	Tr	0.1	0.2	Tr	0.1	Tr
Cholesterol (mg)	4	14	29	5	10	5
Carbohydrate (g)	17	16	11	43	43	17
Total dietary fiber (g)	0	0	0	0	0	0
Calcium (mg)	452	415	274	345	345	325
Iron (mg)	0.2	0.2	0.1	0.2	0.2	0.3
Potassium (g)	579	531	351	440	442	402
Sodium (mg)	174	159	105	132	133	134
Vitamin A (IU)	16	150	279	16	104	0
Thiamin (mg)	0.11	0.10	0.07	0.09	0.08	0.08
Riboflavin (mg)	0.53	0.49	0.32	0.41	0.40	0.37
Niacin (mg)	0.3	0.3	0.2	0.2	0.2	0.2
Ascorbic acid (mg)	2	2	1	2	2	2

Note: Data is for yogurts fortified with nonfat dry milk, except for plain whole milk yogurt.

Tr, trace.

Source: United States Department of Agriculture (2002) and Chandan (2004).

- Conduct a hazard analysis;
- Determine the Critical Control Points (CCPs);
- Establish critical limits (CLs);
- Establish monitoring procedures;
- Establish corrective actions;
- Establish verification procedures; and
- Establish record-keeping and documentation procedures.

Prerequisite Programs

Prior to the implementation of a HACCP Plan, there is a requirement for dairy plants, receiving stations and transfer stations to develop, document, and implement written prerequisite programs (PPs). PPs provide the basic environment and operating conditions that are necessary for the production of safe, wholesome food. Complete, up-to-date process flow diagrams are required for all milk and milk products manufactured. Flow diagrams may be combined when processes, products, and hazards are similar.

HACCP is not a stand-alone program, but is part of a larger control system. PPs are the universal procedures used to control the conditions of the milk plant

environment that contribute to the overall safety of the milk or milk product. They represent the sum of programs, practices, and procedures that must be applied to produce and distribute safe milk and milk products in a clean, sanitary environment. They differ from CCPs in that they are basic sanitation programs that reduce the potential occurrence of a milk or milk product safety hazard. Frequently, both CCPs and PPs control measures are necessary to control a food safety hazard.

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 milk plant, receiving station or transfer station programs or by PPs, as the milk plant, receiving station or transfer station chooses.

The following required PPs shall have a brief written description or checklist that the PPs can be audited against to insure compliance. PPs shall include procedures that can be monitored; records that specify what

Table 1.13. Nutrient Profile of Some Dairy Products

Nutrient	Buttermilk		Sour Cream		Butter, Salted		Cottage Cheese, Low Fat, 2% Fat		Cream Cheese, Regular		Cheddar Cheese		Mozzarella Cheese, Part Skim, Low Moisture	
	1 Cup (245 g)	90	1 Cup (230 g)	71	1 tbsp (14 g)	16	1 Cup (226 g)	79	1 oz (28 g)	54	1 oz (28 g)	37	1 oz (28 g)	49
Moisture (%)	99	90	71	493	102	16	203	79	99	54	114	37	79	49
Calories (kcal)	8	99	7	7	Tr	Tr	31	31	2	2	7	7	8	8
Protein (g)	2	2	48	30.0	7.2	12	4	4	10	10	9	9	5	5
Total fat (g)	1.3	1.3	13.9	1.8	0.4	0.4	2.8	2.8	6.2	6.2	6.0	6.0	3.1	3.1
Saturated fatty acids (g)	0.6	0.6	13.9	1.8	3.3	3.3	1.2	1.2	2.8	2.8	2.7	2.7	1.4	1.4
Monounsaturated fatty acids (g)	0.1	0.1	1.8	1.8	0.4	0.4	0.1	0.1	0.4	0.4	0.3	0.3	0.1	0.1
Polyunsaturated fatty acid (g)	9	9	102	102	31	31	19	19	31	31	30	30	15	15
Cholesterol (mg)	12	12	10	10	Tr	Tr	8	8	1	1	Tr	Tr	1	1
Carbohydrate (g)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total dietary fiber (g)	285	285	268	268	3	3	155	155	23	23	204	204	207	207
Calcium (mg)	0.1	0.1	0.1	0.1	Tr	Tr	0.4	0.4	0.3	0.3	0.2	0.2	0.1	0.1
Iron (mg)	371	371	331	331	4	4	217	217	34	34	28	28	27	27
Potassium (g)	257	257	123	123	117	117	918	918	84	84	176	176	150	150
Sodium (mg)	81	81	1,817	1,817	434	434	158	158	405	405	300	300	199	199
Vitamin A (IU)	0.08	0.08	0.08	0.08	Tr	Tr	0.05	0.05	Tr	Tr	0.01	0.01	0.01	0.01
Thiamin (mg)	0.38	0.38	0.34	0.34	Tr	Tr	0.42	0.42	0.06	0.06	0.11	0.11	0.10	0.10
Riboflavin (mg)	0.1	0.1	0.2	0.2	Tr	Tr	0.3	0.3	Tr	Tr	Tr	Tr	Tr	Tr
Niacin (mg)	2	2	2	2	0	0	0	0	0	0	0	0	0	0
Ascorbic acid (mg)	Tr, trace													

Source: United States Department of Agriculture (2002).

is monitored; and how often it will be monitored. Each milk plant, receiving station or transfer station shall have and implement PPs that address conditions and practices before, during, and after processing. The PPs shall address safety of the water that comes into contact with milk or milk products or product-contact surfaces, including steam and ice; condition and cleanliness of equipment product-contact surface; prevention of cross-contamination from unsanitary objects and/or practices to milk or milk products or product-contact surfaces, packaging material, and other food-contact surfaces, including utensils, gloves, outer garments, and so forth, and from raw product to processed product. Furthermore, PPs involve maintenance of hand washing, hand sanitizing, and toilet facilities; protection of milk or milk product, packaging material, and product-contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate and other chemical, physical, and biological contaminants; proper labeling, storage, and use of toxic compounds; control of employee health conditions, including employee exposure to high-risk situations, that could result in the microbiological contamination of milk or milk products, packaging materials, and product-contact surfaces; and, pest exclusion from the milk plant.

The milk plant, receiving station or transfer station shall monitor the conditions and practices of all required PPs with sufficient frequency to insure conformance with those conditions and that are appropriate both to the milk plant, receiving station or transfer station and to the safety of the milk or milk product being processed. Each milk plant, receiving station or transfer station shall document the correction of those conditions and practices that are not in conformance. Devices, such as indicating and recording thermometers that are used to monitor PPs shall be calibrated to assure accuracy at a frequency determined by the milk plant, receiving station, or transfer station. Each milk plant, receiving station or transfer station shall maintain records that document the monitoring and corrections

Hazard Analysis

Each milk plant, receiving station or transfer station shall develop, or have developed for it, a written hazard analysis to determine whether there are milk or milk product hazards that are reasonably likely to occur for each type of milk or milk product processed or

handled by the milk plant, receiving station or transfer station and to identify the control measures that the milk plant, receiving station or transfer station can apply to control those hazards.

The hazard analysis shall include hazards that can be introduced both within and outside the milk plant, receiving station or transfer station environment, including hazards that can occur during handling, transportation, processing, and distribution.

A hazard that is reasonably likely to occur is one for which a prudent milk plant, receiving station or transfer station operator would establish controls because experience, illness data, scientific reports, or other information provide a basis to conclude that there is a reasonable possibility that, in the absence of these controls, the hazard will occur in the particular type of milk or milk product being processed. In evaluating what milk or milk product hazards are reasonably likely to occur, at a minimum, consideration should be given to microbiological contamination, parasites, chemical contamination, unlawful drug and pesticide residues, natural toxins, unapproved use of food or color additives, presence of undeclared ingredients that may be allergens, and physical hazards. Milk plant, receiving station or transfer station operators should evaluate product ingredients, processing procedures, packaging, storage, and intended use; facility and equipment function and design; and milk plant sanitation, including employee hygiene, to determine the potential effect of each on the safety of the finished milk or milk product for the intended consumer.

HACCP Plan

Every milk plant, receiving station or transfer station shall have and implement a written HACCP plan whenever a hazard analysis reveals one or more hazards that are reasonably likely to occur. A HACCP plan shall be specific to each location and milk or milk product. The plan may group similar types of milk and milk products together, or similar types of production methods together, if the hazards, CCPs, CLs, and procedures required to be identified and performed are essentially identical, provided that any required features of the plan that are unique to a specific milk or milk product or method are clearly delineated in the plan and are observed in practice. The HACCP plan includes complete up-to-date process flow diagrams for all milk and milk products manufactured. Flow diagrams may be combined when processes,

milk and milk products, and hazards are similar. The plan requires:

All hazards that are reasonably likely to occur as identified in the hazard analysis specified above, and that must be controlled for each type of milk or milk product.

The CCPs for each of the identified hazards, including the appropriate CCPs designed to control hazards that could occur or could be introduced in the milk plant, receiving station or transfer station environment are listed. The procedures and the frequencies with which they are to be performed that will be used to monitor each of the CCPs to insure compliance with the CLs. Any corrective action plans that have been developed in accordance with the corrective action requirements and that are to be followed in response to deviations from CLs at CCPs should be included in the plan. Verification and validation procedures, and the frequency with which they are to be performed, are also included. Finally, a record-keeping system that documents the monitoring of the CCPs in accordance with the record requirements must be instituted. The records shall contain the actual values and observations obtained during monitoring.

Sanitation controls may be included in the HACCP plan. However, to the extent that they are monitored in accordance with the PPs, they need not be included in the HACCP Plan.

Corrective Actions

Whenever a deviation from a CL occurs, a milk plant, receiving station or transfer station shall take corrective action. Milk plants, receiving stations or transfer stations may develop written corrective action plans, which become a part of their HACCP plan(s). A corrective action plan that is appropriate for a particular deviation describes the steps to be taken and assigns responsibility for taking those steps, to insure that no milk or milk product is allowed to enter commerce that is either injurious to health or is otherwise adulterated as a result of the deviation, or if such milk or milk product has entered commerce, it is expeditiously removed; and the cause of the deviation is corrected. Verification activities shall include the calibration of CCP process-monitoring instruments, verification also includes a review, including signing and dating, by an individual records that document to insure that the records are complete and to

verify that the recorded document values are within the CLs.

Validation of the HACCP Plan

Every milk plant, receiving station or transfer station shall validate that the HACCP plan is adequate to control hazards that are reasonably likely to occur. This validation shall occur at least once within 12 months after implementation and at least annually thereafter or whenever any changes in the process occur that could affect the hazard analysis or alter the HACCP plan.

Required Records

It is essential that milk plants, receiving stations, and transfer stations use consistent terminology to identify each piece of equipment, record, document, or other program throughout their written HACCP system. All records shall be retained at the milk plant, receiving station or transfer station for perishable or refrigerated products, for at least 1 year after the date that such products were prepared, and in the case of frozen, preserved, or shelf-stable products, for 2 years after the date that the products were prepared or the shelf life of the product, whichever is greater, unless longer retention time is required by other regulations.

Training and Standardization

Regulatory agency personnel responsible for the evaluation, licensing, and regulatory audits of facilities using the HACCP program will have specialized training in conducting HACCP system audits. Industry, State, and Federal regulatory and listing personnel should be trained together.

HACCP Audits and Follow-up Actions

Audits shall be conducted of the milk plant, receiving station, or transfer station facility, and HACCP program to insure compliance with the HACCP system. The audit may be announced at the discretion of the auditor under certain circumstances, that is, initial audit, follow-up audit, new construction, pasteurizer checks, and so forth. When unannounced audits are conducted, the audits shall not be completed until appropriate milk plant personnel have had an opportunity to make all pertinent records available for review by the auditor.

After initial audit, the next audit is 30–45 days and at 4-month interval thereafter. Next audits are normally scheduled 6 months later. The regulatory agency has the discretion of auditing with greater frequency. In addition, compliance follow-ups are made to insure that the observed problems have been resolved.

Chapter 22 discusses in detail the management systems for safety and quality of milk and dairy products. Chapter 23 deals with the laboratory analysis associated with quality assurance programs.

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2

Dairy Industry: Production and Consumption Trends

Ramesh C. Chandan

Introduction

Overview of The World Dairy Industry

World Milk Production

World Production of Cow's Milk and Milk Products

Dairy Processing Industry in The United States

Trends in the U.S. Milk Production

Trends in the Size and Number of Dairy Manufacturing

Plants in the United States

Trends in the Sales of Milk Products

Per Capita Consumption

References

INTRODUCTION

Milk has been recognized as a staple food for humans for thousands of years. This chapter contains information related to the production and consumption patterns of milk and dairy foods. An overview of the world dairy industry is followed by an overview of dairy scenario in the United States.

OVERVIEW OF THE WORLD DAIRY INDUSTRY

Various regions of the world utilize milk of different milch animals for human consumption (Chandan 2006). Although cow's milk dominates in human nutrition, milk of water buffalo, goat, sheep, camel, and yak has an important role in several countries. South Asia, China, Egypt, and Philippines are the home of milk of water buffaloes. The market milk available in India and Pakistan contains a significant percentage of buffalo's milk commingled with cow's milk. The chemical composition and sensory attributes of milk of various species differ appreciably from each other but form an integral part of cultural ethos of the

consumers. Accordingly, consumers in various parts of the world enjoy the attributes of mouth feel, flavor profile texture, and appearance of the milk dominant in their countries. The sensory characteristic, therefore, is the norm for them whereas it may be objectionable for a consumer unfamiliar with the taste and flavor of milk of particular species. It is interesting to observe that Mozzarella cheese made from buffalo's milk, Roquefort cheese made from sheep's milk, and several cheeses made from goat's milk possess characteristic appearance or flavor giving them distinctive position and price advantage among the dairy foods of the world.

The chemical composition of various milks is given in Table 2.1 to demonstrate wide differences in total solids, protein, fat, and other major constituents among them.

The total solids content is highest in the milk of sheep, followed by yak, buffalo, zebu cow, camel, goat, cow, mare, and donkey. Wide variations in total solids are evident. Sheep's milk has 19.3% total solids while donkey's milk has the lowest at 8.5%. Sheep's milk has the highest fat content at 7.3%, followed by buffalo's milk at 6.7%. Donkey's milk has only 0.6% fat and mare's milk has 1.9% fat. The total protein content varies from 1.4% in donkey's milk to 5.8% in yak's milk. Milk of sheep has the highest casein content whereas milk of mare and donkey has the lowest casein content. Milk of mare has the highest whey protein content, followed by milk of buffalo, sheep, and camel. Milk of mare and donkey has the highest lactose content. Ash content of milk is a crude measure of its mineral content. Donkey's milk is low in ash content while sheep's and yak's milks are rich in ash content. Goat's and buffalo's milks have more ash content than cow's milk.

Table 2.1. Proximate Composition of Milk of Mammals Used for Human Consumption

Mammal	% Total Solids	% Fat	% Total Protein	% Casein	% Whey Protein	% Lactose	% Ash
Cow	12.2	3.4	3.4	2.8	0.6	4.7	0.7
Cow, zebu	13.8	4.6	3.3	2.6	0.7	4.4	0.7
Buffalo	16.3	6.7	4.5	3.6	0.9	4.5	0.8
Goat	13.2	4.5	2.9	2.5	0.4	4.1	0.8
Sheep	19.3	7.3	5.5	4.6	0.9	4.8	1.0
Camel	13.6	4.5	3.6	2.7	0.9	5.0	0.7
Mare	11.2	1.9	2.5	1.3	1.2	6.2	0.5
Donkey	8.5	0.6	1.4	0.7	0.7	6.1	0.4
Yak	17.3	6.5	5.8	NA	NA	4.6	0.9

NA, not available.

Source: Adapted from Chandan et al. (2006).

Accordingly, the technology for making dairy products from milk of various species is strikingly different.

WORLD MILK PRODUCTION

Table 2.2 shows the milk output in several countries.

The world production of milk of all species was estimated to be 640.2 million metric tons (MT) (1,411,385 million pounds) in 2005, which registered a growth of 2.4% over the production during 2004 (Cluff and Senfter, 2007). Valuable information on world dairy production is available from Food and Agricultural Organization of the United Nations in Rome (<http://faostat.fao.org>). The growth is primarily attributed to the growth in developing countries in Asia and South America. The 25 countries constituting European Union produced 147.4 million MT (324,958 million pounds) in 2005. As a country, India is the largest producer of milk with 95.4 million MT (210,319 million pounds), followed by the U.S. output of 80.6 MT (177,691 million pounds). China has registered the largest growth rate in recent years. The gap in production and consumption is the key to import–export demand and dairy trade around the world. Nonfat dry milk and whole milk powder constitute almost 50% of international dairy trade. The highest growth in milk powder imports has been observed in the countries located in Southeast Asia, particularly China and Philippines. Mexico, Algeria, and Morocco are also significant importers of milk powders.

Butter and cheese form a significant part of the trade. Russian Federation, countries in North Africa, and Near East are the key markets for importing butter. Japan is the world's largest cheese importer.

New Zealand is the leading exporter of dairy products. Other significant exporting countries are

Australia, Argentina, Brazil, Colombia, Chile, the United States, and the EU countries. The trade situation at a given time is driven by international demand, prices, surplus stocks, governmental subsidies, currency exchange rates, and income growth particularly related to oil export revenues.

WORLD PRODUCTION OF COW'S MILK AND MILK PRODUCTS

Number of Cows

The estimated number of cows in 2007 was 125 million heads (Table 2.3). The milk yield of individual cow varies widely in the world. In 2007, the United States was projected to be the most efficient milk producer with 9.37 MT (20,661 pounds) per cow, followed by the Japanese cow yield of 9.04 MT (19,933 pounds) per cow. Fluid milk production in India, Mexico, and Brazil is among the lowest at 1.04–1.69 MT (2,293–3,726 pounds) per head.

Production and Consumption of Fluid Milk and Other Dairy Products

The world production of cow's milk in the year 2007 was projected at 427 million MT (942,664 million pounds; Table 2.4). Cow's milk accounts for approximately two thirds of all the milk production in the world. In 2007, the production of fluid milk in the countries of the European Union was estimated at 131 million MT (289,957 million pounds), while as a country the largest producer of cow's milk is the United States producing 85 million MT (188,406 million pounds), followed by India at 40 million MT (88,310 million pounds) and China at 38 million MT (84,010 million pounds). The top consuming

Table 2.2. Total Milk Production (All Animals) in Major Milk Producing Countries (2003–2005)

Countries	2003	2004 ^p	2005 ^f	% Growth (2005/2004)
EU-25				
Million MT	147.6	146.4	147.4	0.7
<i>Million pounds</i>	325,399	322,753	324,958	
India				
Million MT	87.3	91.1	95.4	4
<i>Million pounds</i>	192,462	200,839	210,319	
United States				
Million MT	77.3	77.5	80.6	4.0
<i>Million pounds</i>	170,416	170,856	177,691	
Russian Federation				
Million MT	33.4	32.2	32.2	0.0
<i>Million pounds</i>	73,634	70,988	70,988	
Pakistan				
Million MT	27.8	28.6	29.5	3.2
<i>Million pounds</i>	61,288	63,052	65,036	
Brazil				
Million MT	23.5	23.7	24.6	3.8
<i>Million pounds</i>	51,808	52,249	54,233	
China				
Million MT	21.5	27.1	32.5	20.0
<i>Million pounds</i>	47,399	59,745	71,650	
New Zealand				
Million MT	14.4	15.0	14.6	−2.7
<i>Million pounds</i>	31,746	33,069	32,187	
Ukraine				
Million MT	13.7	13.6	13.6	0.0
<i>Million pounds</i>	30,203	29,983	29,983	
Mexico				
Million MT	9.9	10.0	10.1	1.2
<i>Million pounds</i>	21,826	22,046	22,266	
Argentina				
Million MT	8.2	9.6	10.1	4.8
<i>Million pounds</i>	18,078	21,164	22,266	
Turkey				
Million MT	10.6	10.5	10.5	0.2
<i>Million pounds</i>	23,369	23,148	23,148	
Australia				
Million MT	10.3	10.1	10.1	0.0
<i>Million pounds</i>	22,707	22,266	22,266	
Japan				
Million MT	8.4	8.4	8.3	−0.6
<i>Million pounds</i>	18,519	18,519	18,298	
Canada				
Million MT	7.7	7.9	7.8	−1.4
<i>Million pounds</i>	16,975	17,416	17,196	
World				
Million MT	615.5	625.2	640.2	2.4
<i>Million pounds</i>	1,356,931	1,378,316	1,411,385	

MT, metric tons; p, provisional; f, forecast.

Source: Cluff and Senfter (2007).

Table 2.3. Milk Cows and Milk Yield per Cow in Selected Countries in 2005–2007

	Milk Cows (1,000 Heads) in the Year			Milk Yield/Cow in the Year			
Country	2005	2006 ^p	2007 ^f		2005	2006 ^p	2007 ^f
Canada	1,066	1,049	1,029	MT	7.32	7.41	7.43
				<i>Pounds</i>	<i>16,141</i>	<i>16,339</i>	<i>16,383</i>
Mexico	6,850	6,875	6,885	MT	1.44	1.46	1.47
				<i>Pounds</i>	<i>3,109</i>	<i>3,219</i>	<i>3,241</i>
United States	9,045	9,112	9,120	MT	8.87	9.05	9.37
				<i>Pounds</i>	<i>19,558</i>	<i>19,955</i>	<i>20,661</i>
Argentina	2,100	2,150	2,180	MT	4.52	4.79	4.95
				<i>Pounds</i>	<i>9,967</i>	<i>10,562</i>	<i>10,915</i>
Brazil	15,100	15,050	15,020	MT	1.59	1.64	1.69
				<i>Pounds</i>	<i>3,506</i>	<i>3,616</i>	<i>3,726</i>
European Union	23,400	22,970	22,340	MT	5.63	5.68	5.89
				<i>Pounds</i>	<i>12,414</i>	<i>12,524</i>	<i>12,987</i>
Russia	10,400	9,900	9,910	MT	3.08	3.14	3.23
				<i>Pounds</i>	<i>6,791</i>	<i>6,924</i>	<i>7,122</i>
Ukraine	4,130	3,840	3,800	MT	3.25	3.36	3.45
				<i>Pounds</i>	<i>7,126</i>	<i>7,409</i>	<i>7,607</i>
India	38,000	38,000	38,500	MT	0.99	1.02	1.04
				<i>Pounds</i>	<i>2,183</i>	<i>2,249</i>	<i>2,293</i>
China	6,800	8,100	9,300	MT	4.05	4.05	4.10
				<i>Pounds</i>	<i>8,930</i>	<i>8,930</i>	<i>9,040</i>
Japan	910	900	895	MT	9.10	9.04	9.04
				<i>Pounds</i>	<i>20,065</i>	<i>19,933</i>	<i>19,933</i>
Australia	2,041	2,045	1,950	MT	5.11	5.08	5.02
				<i>Pounds</i>	<i>11,268</i>	<i>11,201</i>	<i>11,069</i>
New Zealand	3,970	4,100	4,140	MT	3.65	3.71	3.77
				<i>Pounds</i>	<i>8,048</i>	<i>8,181</i>	<i>8,313</i>
Total selected countries	123,812	124,091	125,069				

MT, metric tons; p, preliminary; f, forecast.

Source: http://www.fas.usda.gov/dlp/circular/2007/dairy_07-2007.pdf.

countries are India at 42 million MT (92,334 million pounds), followed by the EU countries at 34 million MT (75,080 million pounds) and the United States at 27 million MT (60,402 million pounds). The data given here correspond to consumption of milk in fluid form and differences between production volumes and consumption values may be due to the conversion and consumption of fluid milk into butter, cheese, yogurt, ice cream, and other dairy products.

Production and Consumption of Butter, Cheese, and Milk Powders

Table 2.5 shows the butter production and consumption in the selected countries of the world.

During 2007, India was estimated to produce more butter than any other country (3 million MT, or 7,551

million pounds), followed by the European Union at 2 million MT (4,519 million pounds) and the United States at 0.6 million MT (1,446 million pounds). On the consumption side, India is the largest consumer of butter at 3 million MT (7,540 million pounds), followed by the European Union at 2 million MT (4,303 million pounds) and the United States at 0.9 million MT (1,993 million pounds). The disparity between production volume and consumption of butter in a given country is indicative of import–export opportunity. For example, New Zealand has a surplus of approximately 3.9 million MT of butter per year. The consumption pattern of butter in Mexico and Russia leaves a deficit to be satisfied with import of butter from surplus countries.

Cheese production and consumption patterns are shown in Table 2.6.

Table 2.4. Fluid Cow's Milk Production and Consumption in Selected Countries in 2005–2007

Country	2005		2006 ^p		2007 ^f	
	Production	Consumption	Production	Consumption	Production	Consumption
Canada						
1,000 MT	7,806	2,831	7,773	2,823	7,650	2,778
<i>Million pounds</i>	26,242	17,21	17,139	6,225	18,868	6,125
Mexico						
1,000 MT	9,855	4,266	10,051	4,305	10,100	4,344
<i>Million pounds</i>	21,730	9,407	22,163	9,493	22,270	9,579
United States						
1,000 MT	80,253	27,231	82,462	27,310	85,445	27,393
<i>Million pounds</i>	176,953	60,044	181,829	60,219	188,406	60,402
Argentina						
1,000 MT	9,500	1,800	10,300	1,900	10,800	1,960
<i>Million pounds</i>	20,947	3,969	22,711	4,189	23,814	4,322
Brazil						
1,000 MT	24,025	13,175	24,745	13,309	25,365	13,445
<i>Million pounds</i>	52,975	29,051	54,563	29,346	55,930	29,646
European Union						
1,000 MT	131,652	34,064	130,400	34,030	131,500	34,050
<i>Million pounds</i>	290,293	75,111	287,532	75,036	289,957	75,080
Russia						
1,000 MT	32,000	12,850	31,100	12,000	32,000	12,000
<i>Million pounds</i>	70,560	28,334	68,575	26,460	70,560	26,460
Ukraine						
1,000 MT	13,423	5,441	12,890	6,086	13,100	6,219
<i>Million pounds</i>	29,598	11,997	28,422	13,420	28,885	13,713
India						
1,000 MT	37,520	36,600	38,750	38,840	40,050	41,875
<i>Million pounds</i>	82,732	80,703	85,444	85,642	88,310	92,334
China						
1,000 MT	27,534	12,500	32,800	14,750	38,100	16,900
<i>Million pounds</i>	60,712	27,562	72,324	32,524	84,010	37,264
Japan						
1,000 MT	8,285	4,775	8,134	4,645	8,090	4,550
<i>Million pounds</i>	18,268	10,529	17,935	10,242	17,838	10,033
Australia						
1,000 MT	10,429	2,145	10,395	2,127	9,785	2,000
<i>Million pounds</i>	22,996	4,730	22,921	4,690	21,575	4,410
New Zealand						
1,000 MT	14,500	360	15,200	360	15,600	360
<i>Million pounds</i>	31,972	794	33,516	794	34,398	794
Total selected countries						
1,000 MT	406,782	158,038	415,000	162,485	427,585	67,874
<i>Million pounds</i>	896,801	348,414	914,918	58,218	942,664	370,099

MT, metric tons; p, preliminary; f, forecast.

Source: http://www.fas.usda.gov/dlp/circular/2007/dairy_07-2007.pdf.

Table 2.5. Butter Production and Consumption in Selected Countries in 2005–2007

Country	2005		2006 ^p		2007 ^f	
	Production	Consumption	Production	Consumption	Production	Consumption
Canada						
1,000 MT	84	83	81	88	82	88
<i>Million pounds</i>	<i>185</i>	<i>183</i>	<i>179</i>	<i>194</i>	<i>181</i>	<i>194</i>
Mexico						
1,000 MT	93	144	109	158	110	160
<i>Million pounds</i>	<i>205</i>	<i>317</i>	<i>240</i>	<i>348</i>	<i>243</i>	<i>353</i>
United States						
1,000 MT	611	838	657	887	656	904
<i>Million pounds</i>	<i>1,347</i>	<i>1,847</i>	<i>1,448</i>	<i>1,956</i>	<i>1,446</i>	<i>1,993</i>
Argentina						
1,000 MT	788	NA	847	NA	848	NA
<i>Million pounds</i>	<i>1,737</i>	<i>NA</i>	<i>1,867</i>	<i>NA</i>	<i>1,870</i>	<i>NA</i>
Brazil						
1,000 MT	77	77	78	77	79	78
<i>Million pounds</i>	<i>170</i>	<i>170</i>	<i>172</i>	<i>170</i>	<i>174</i>	<i>172</i>
European Union						
1,000 MT	2,140	1,924	2,055	1,948	2,050	1,952
<i>Million pounds</i>	<i>4,718</i>	<i>4,242</i>	<i>4,530</i>	<i>4,295</i>	<i>4,519</i>	<i>4,303</i>
Russia						
1,000 MT	275	385	275	400	300	420
<i>Million pounds</i>	<i>606</i>	<i>849</i>	<i>606</i>	<i>882</i>	<i>661</i>	<i>926</i>
Ukraine						
1,000 MT	118	94	105	92	110	90
<i>Million pounds</i>	<i>260</i>	<i>207</i>	<i>231</i>	<i>203</i>	<i>243</i>	<i>198</i>
India						
1,000 MT	2,749	2,743	3,050	3,045	3,425	3,420
<i>Million pounds</i>	<i>6,061</i>	<i>6,047</i>	<i>6,724</i>	<i>6,713</i>	<i>7,551</i>	<i>7,540</i>
Japan						
1,000 MT	84	86	80	89	82	89
<i>Million pounds</i>	<i>185</i>	<i>190</i>	<i>176</i>	<i>196</i>	<i>181</i>	<i>196</i>
Australia						
1,000 MT	131	65	129	62	115	60
<i>Million pounds</i>	<i>289</i>	<i>143</i>	<i>284</i>	<i>137</i>	<i>254</i>	<i>132</i>
New Zealand						
1,000 MT	340	26	390	26	419	26
<i>Million pounds</i>	<i>750</i>	<i>57</i>	<i>860</i>	<i>57</i>	<i>924</i>	<i>57</i>

MT, metric tons; p, preliminary; f, forecast; NA, not available.

Source: http://www.fas.usda.gov/dlp/circular/2007/dairy_07-2007.pdf.

Cheese production in 2007 was highest in the EU countries at 7 million MT (14,771 million pounds), followed by the United States at 4 million MT (9,727 million pounds) and Brazil at 0.5 million MT (1,113 million pounds). The consumption figure show surplus in New Zealand, Australia, Argentina, the EU countries, and Ukraine while the demand exceeds production in Japan, Russia, Canada, and Korea.

Table 2.7 shows the production and consumption of nonfat dry milk in selected countries.

The projected leaders in the production of nonfat dry milk in 2007 were the EU countries with 0.975 million MT (2,150 million pounds), the United States with 0.652 million MT (1,437 million pounds), and India with 0.34 million MT (750 million pounds). The surplus countries are New Zealand, Australia,

Table 2.6. Cheese Production and Consumption in Selected Countries in 2005–2007

Country	2005		2006 ^p		2007 ^f	
	Production	Consumption	Production	Consumption	Production	Consumption
Canada						
1,000 MT	352	365	50	366	351	367
<i>Million pounds</i>	776	805	772	807	774	809
Mexico						
1,000 MT	143	230	145	229	147	233
<i>Million pounds</i>	315	507	320	505	324	514
United States						
1,000 MT	4,150	4,869	4,325	5,025	4,412	5,110
<i>Million pounds</i>	9,150	10,734	9,535	11,078	9,727	11,266
Argentina						
1,000 MT	460	405	475	420	488	440
<i>Million pounds</i>	1,014	893	1,047	926	1,076	910
Brazil						
1,000 MT	480	472	495	490	505	501
<i>Million pounds</i>	1,058	1,041	1,091	1,080	1,113	1,105
European Union						
1,000 MT	6,480	6,083	6,580	6,152	6,700	6,250
<i>Million pounds</i>	14,286	13,411	14,506	13,563	14,771	13,779
Russia						
1,000 MT	375	615	405	625	420	660
<i>Million pounds</i>	827	1,356	893	1,378	926	1,455
Ukraine						
1,000 MT	274	164	210	170	200	150
<i>Million pounds</i>	604	362	463	375	441	331
Japan						
1,000 MT	39	251	39	246	42	247
<i>Million pounds</i>	86	553	86	542	93	545
Korea						
1,000 MT	24	69	24	69	26	73
<i>Million pounds</i>	53	152	53	152	57	161
Philippines						
1,000 MT	5	NA	6	NA	7	NA
<i>Million pounds</i>	11	NA	13	NA	15	NA
Taiwan						
1,000 MT	16	16	18	18	20	20
<i>Million pounds</i>	35	35	40	40	44	44
Australia						
1,000 MT	375	223	362	220	360	215
<i>Million pounds</i>	827	492	798	485	794	474
New Zealand						
1,000 MT	297	28	285	28	319	28
<i>Million pounds</i>	655	62	628	62	703	62

MT, metric tons; p, preliminary; f, forecast; NA, not available.

Source: http://www.fas.usda.gov/dlp/circular/2007/dairy_07-2007.pdf.

Table 2.7. Nonfat Dry Milk Production and Consumption in Selected Countries in 2005–2007

Country	2005		2006 ^p		2007 ^f	
	Production	Consumption	Production	Consumption	Production	Consumption
Canada						
1,000 MT	73	74	66	66	64	56
<i>Million pounds</i>	<i>161</i>	<i>163</i>	<i>146</i>	<i>146</i>	<i>141</i>	<i>123</i>
Mexico						
1,000 MT	155	311	183	294	190	300
<i>Million pounds</i>	<i>342</i>	<i>686</i>	<i>403</i>	<i>648</i>	<i>419</i>	<i>661</i>
United States						
1,000 MT	695	957	686	795	652	759
<i>Million pounds</i>	<i>1,532</i>	<i>2,110</i>	<i>1,512</i>	<i>1,753</i>	<i>1,437</i>	<i>1,673</i>
Argentina						
1,000 MT	32	14	32	14	34	14
<i>Million pounds</i>	<i>71</i>	<i>31</i>	<i>71</i>	<i>31</i>	<i>75</i>	<i>31</i>
Brazil						
1,000 MT	113	128	117	136	125	144
<i>Million pounds</i>	<i>249</i>	<i>282</i>	<i>258</i>	<i>300</i>	<i>276</i>	<i>317</i>
European Union						
1,000 MT	1,065	947	975	914	975	895
<i>Million pounds</i>	<i>2,348</i>	<i>2,088</i>	<i>2,150</i>	<i>2,015</i>	<i>2,150</i>	<i>1,973</i>
Russia						
1,000 MT	110	165	110	140	115	150
<i>Million pounds</i>	<i>243</i>	<i>364</i>	<i>243</i>	<i>309</i>	<i>254</i>	<i>331</i>
Ukraine						
1,000 MT	78	21	80	15	85	15
<i>Million pounds</i>	<i>172</i>	<i>46</i>	<i>176</i>	<i>33</i>	<i>187</i>	<i>33</i>
China						
1,000 MT	60	101	55	119	55	134
<i>Million pounds</i>	<i>132</i>	<i>223</i>	<i>121</i>	<i>262</i>	<i>121</i>	<i>295</i>
India						
1,000 MT	256	225	295	240	340	280
<i>Million pounds</i>	<i>564</i>	<i>496</i>	<i>650</i>	<i>529</i>	<i>750</i>	<i>617</i>
Japan						
1,000 MT	187	227	181	226	185	225
<i>Million pounds</i>	<i>412</i>	<i>500</i>	<i>399</i>	<i>498</i>	<i>408</i>	<i>496</i>
Korea						
1,000 MT	24	30	23	31	23	29
<i>Million pounds</i>	<i>53</i>	<i>66</i>	<i>51</i>	<i>68</i>	<i>51</i>	<i>64</i>
Philippines						
1,000 MT	NA	74	NA	76	NA	79
<i>Million pounds</i>	<i>NA</i>	<i>163</i>	<i>NA</i>	<i>168</i>	<i>NA</i>	<i>174</i>
Taiwan						
1,000 MT	NA	19	NA	19	NA	18
<i>Million pounds</i>	<i>NA</i>	<i>42</i>	<i>NA</i>	<i>42</i>	<i>NA</i>	<i>40</i>
Australia						
1,000 MT	206	35	221	35	200	44
<i>Million pounds</i>	<i>454</i>	<i>77</i>	<i>487</i>	<i>77</i>	<i>441</i>	<i>97</i>
New Zealand						
1,000 MT	225	40	247	40	304	49
<i>Million pounds</i>	<i>496</i>	<i>88</i>	<i>545</i>	<i>88</i>	<i>670</i>	<i>108</i>

MT, metric tons; p, preliminary; f, forecast; NA, not available.

Source: http://www.fas.usda.gov/dlp/circular/2007/dairy_07-2007.pdf.

Table 2.8. Whole Milk Powder Production and Consumption in Selected Countries in 2005–2007

Country	2005		2006 ^p		2007 ^f	
	Production	Consumption	Production	Consumption	Production	Consumption
United States						
1,000 MT	15	18	14	16	17	17
<i>Million pounds</i>	33	40	31	35	37	37
Argentina						
1,000 MT	255	98	295	102	310	110
<i>Million pounds</i>	562	216	650	225	683	243
Brazil						
1,000 MT	440	449	465	469	485	500
<i>Million pounds</i>	970	990	1,025	1,034	1,069	1,102
European Union						
1,000 MT	858	367	810	382	805	377
<i>Million pounds</i>	1,892	809	1,786	842	1,775	831
Russia						
1,000 MT	85	110	90	100	95	115
<i>Million pounds</i>	187	243	198	220	209	254
Ukraine						
1,000 MT	28	8	30	10	32	10
<i>Million pounds</i>	62	18	66	22	71	22
China						
1,000 MT	918	951	1,030	1,081	1,150	1,214
<i>Million pounds</i>	2,024	2,097	2,271	2,383	2,535	2,676
Indonesia						
1,000 MT	48	75	48	74	47	75
<i>Million pounds</i>	106	165	106	165	104	165
Philippines						
1,000 MT	NA	31	NA	30	NA	30
<i>Million pounds</i>	NA	68	NA	66	NA	66
Taiwan						
1,000 MT	5	31	6	31	6	30
<i>Million pounds</i>	11	68	13	66	13	66
Australia						
1,000 MT	189	22	158	27	140	27
<i>Million pounds</i>	417	49	348	59	309	59
New Zealand						
1,000 MT	585	1	634	1	655	1
<i>Million pounds</i>	1,290	3	1,398	2	1,444	2

MT, metric tons; p, preliminary; f, forecast; NA, not available.

Source: http://www.fas.usda.gov/dlp/circular/2007/dairy_07-2007.pdf.

Ukraine, Argentina, and India. The countries consuming more nonfat dry milk than production volumes are Mexico, Russia, China, and Japan. Nonfat dry milk production is related to the surplus of milk left over from the conversion of milk to other dairy products. Accordingly, its production varies a lot from year to year.

In case of whole milk powder, China leads production at 1,150 million MT (2,535 million

pounds), followed by the EU countries at 0.805 million MT (1,775 million pounds) and New Zealand at 0.655 MT (1,444 million pounds; see Table 2.8).

The countries in a position to export are EU countries, Ukraine, and Argentina. The countries likely to import are Brazil, Russia, China, Indonesia, and Taiwan. The production volumes do change from year to year.

Table 2.9. Per Capita Consumption of Various Dairy Products by Selected Countries in 2005

Country	Fluid Milk		Cheese		Butter		Nonfat Dry Milk		Whole Milk Powder	
	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds
Canada	41.4	91.3	11.1	24.5	2.5	5.6	2.3	5.1	NA	NA
Mexico	40.2	88.6	2.1	4.6	1.3	2.9	3.2	7.1	0.3	0.7
United States	92.5	204.0	14.5	32.0	2.1	4.6	2.0	4.5	0.05	0.1
Argentina	48.0	105.9	8.8	19.5	NA	NA	0.5	1.1	2.3	5.0
Brazil	70.8	156.1	2.5	5.6	0.4	0.9	0.6	1.4	2.4	5.3
Peru	0.0	0.0	NA	NA	NA	NA	0.2	0.5	NA	NA
Colombia	NA	NA	NA	NA	NA	NA	0.2	0.5	NA	NA
Chile	NA	NA	NA	NA	NA	NA	0.1	2.1	3.3	7.3
European Union	74.5	164.2	13.4	29.5	4.3	9.4	2.1	4.7	0.8	1.7
Russia	90.0	198.4	4.3	9.5	2.7	5.9	1.3	2.6	0.8	1.7
Ukraine	92.0	202.8	3.5	7.6	2.4	5.2	0.3	0.7	0.2	0.5
Romania	165.7	365.3	1.2	2.6	0.5	1.0	NA	NA	NA	NA
Egypt	NA	NA	6.0	13.2	0.5	1.2	0.3	0.6	NA	NA
Algeria	NA	NA	NA	NA	0.5	1.0	3.0	6.7	4.8	10.7
China	9.9	21.9	NA	NA	NA	NA	0.1	0.2	0.7	1.6
India	35.2	77.6	NA	NA	2.6	5.7	0.2	0.5	NA	NA
Indonesia	NA	NA	NA	NA	NA	NA	0.5	1.2	0.3	0.7
Philippines	NA	NA	NA	NA	NA	NA	1.3	2.8	0.2	0.4
Korea	NA	NA	0.9	2.0	NA	NA	0.4	1.0	NA	NA
Japan	37.5	82.6	2.0	4.3	0.7	1.5	1.8	3.9	NA	NA
Taiwan	NA	NA	NA	NA	0.5	1.2	0.6	1.4	1.4	3.1
Australia	103.7	228.6	11.1	24.5	3.2	7.1	1.0	2.2	1.2	2.7
New Zealand	89.2	196.7	6.9	15.3	6.4	14.2	1.2	2.7	0.3	0.6

NA, not available.

Source: Dairy Facts, 2006 edition. International Dairy Foods Association, Washington, DC.

Per Capita Consumption of Dairy Products in the World

Per capita consumption is a good parameter of how much of each product is consumed in a country and a comparison can be made for various countries in product consuming patterns (Table 2.9).

Fluid milk consumption is the largest in Romania, followed by Australia and the United States. For cheese, per capita consumption is the highest in the United States, followed by the EU countries, Australia, and Canada. Butter consumption, per capita, is the largest in New Zealand, followed by the EU countries and Australia. Per capita consumption of nonfat dry milk is highest in Mexico, followed by Algeria and Canada. Whole milk powder consumption on per capita basis is highest in Algeria, followed by Chile and Brazil.

DAIRY PROCESSING INDUSTRY IN THE UNITED STATES

In the United States, dairy farmers and dairy processors alike abide by the strict state and federal sanitary

standards. Grade "A" Pasteurized Milk Ordinance (PMO) regulations are basically the recommendations of the Public Health Service of the Food and Drug Administration of United States Department of Health and Human Services (DHHS, 1999). The PMO is meant for voluntary adoption but its importance to insure the quality and safety of milk supply in the country is recognized by the dairy industry as well as by the state regulatory and sanitation officials. The PMO is constantly evolving set of regulations to accommodate advancements and developments in science and technology related to milk production, processing, packaging, and distribution. From time to time, modification in the regulations are adopted following an agreement among the representatives of government, industry (milk producers, processors, equipment manufacturers, and suppliers), and academia and research institutions. To conform to PMO, dairy farms and dairy plants are visited regularly by representatives of government regulatory agencies, who conduct quality assurance and safety inspections at the farms and processing plants. These inspectors confirm herd health,

Table 2.10. Milk Production and Its Efficiency in the United States

Year	Milk Cows (1,000 Head)	Production Per Cow		Total Milk Production	
		MT	Pounds	Million MT	Million pounds
1995	9,466	7.44	16,405	70.44	155,292
1996	9,372	7.45	16,433	69.86	154,006
1997	9,252	7.65	16,871	70.80	156,091
1998	9,154	7.79	17,189	71.33	157,348
1999	9,156	8.06	17,772	73.75	162,716
2000	9,206	8.25	18,201	75.93	167,559
2001	9,114	8.24	18,159	74.99	165,497
2002	9,139	8.44	18,608	77.14	170,063
2003	9,084	8.51	18,749	77.29	170,312
2004	9,010	8.60	18,957	77.48	170,805
2005	9,041	8.88	19,576	80.28	176,989
2006 ^p	9,112	9.05	19,955	82.46	181,797
2007 ^f	9,120	9.37	20,657	85.44	188,373

MT, metric tons; p, preliminary; f, forecast.

Source: http://www.fas.usda.gov/dlp/circular/2007/dairy_07-2007.pdf.

oversee veterinary practices, monitor sanitation of the facilities and milking equipment, and verify that the milk is being rapidly cooled and properly stored until delivered to processing facilities. They also insure that the processing of milk is in accordance with the state and federal food laws. In some instances, the state standards differ and may be even more stringent than the federal standards. The state and in some cases local communities have jurisdiction for standards for milk in their own market.

The PMO define Grade A specification and standards for milk and milk products to facilitate movement of milk across state lines. Market milk, cream, yogurt, cultured buttermilk, and sour cream are governed by the Grade A standards. Reciprocity rights maintain that milk conforming to the PMO sanitary standards in one state would not require further inspections for acceptance by another state. The reader is referred to see Chapter 6 for detailed discussion of this topic.

TRENDS IN THE U.S. MILK PRODUCTION

At the dairy farms, modern milking and milk handling equipment, including automated milking systems, have improved the speed of cleaning, sanitizing, cooling, and delivering good quality raw milk to processing plants. The U.S. farm milk production had reached record-high level in 2007 (188.4 billion pounds). The average herd size was 115 and an increase in the number of farms with 500-plus cows registered increase from previous years (International Dairy Foods Association, 2006). The demand for

milk to make cheese has been sharply increasing. In 2005, 39.6% of the total milk produced was used for cheese making. Fluid milk and related operations used 12.9% of the milk supply, while butter manufacture used 12.9% and ice cream and frozen desserts used 8.1%.

During the last decades, the trend indicates decrease in dairy cow population and increase in milk production per cow (Table 2.10).

In the year 2007, 9.12 million cows were projected to produce 85.44 million MT (188,373 million pounds). The table also shows that, in general, there is a steady increase in milk production per cow. Approximately, 20% of the world's milk is produced in the United States. The American dairy farmer has been able to achieve its current milk output through the application of scientific and management advancements to milk production. On the dairy farm, selection of dairy cows, their breeding, and judicious use of balanced feed rations have been instrumental in increasing milk output per cow. In the year 2007, milk production per cow was projected to increase to 9.37 MT (20,657 pounds). As a result of continuous efficiency in milk production at the farm, milk production per cow has doubled in the last 30 years.

TRENDS IN THE SIZE AND NUMBER OF DAIRY MANUFACTURING PLANTS IN THE UNITED STATES

As a country, the United States has the distinction of being top processor of milk and dairy products

Table 2.11. Sales of Milk and Major Retail Dairy Products in the United States During 2000–2005

Product	Sales Volume					
	Year					
	2000	2001	2002	2003	2004	2005
<i>Whole white milk</i>						
1,000 MT	8,368	8,168	8,146	8,088	7,890	7,612
Million pounds	18,448	18,007	17,960	17,832	17,395	16,781
<i>Low-fat/reduced fat white milk</i>						
1,000 MT	10,847	10,727	10,718	10,709	10,686	10,710
Million pounds	23,913	23,649	23,630	23,610	23,559	23,611
<i>Nonfat white milk</i>						
1,000 MT	3,826	3,758	3,642	3,533	3,535	3,626
Million pounds	8,435	8,285	8,030	7,789	7,794	7,994
<i>Flavored milk and drinks</i>						
1,000 MT	1,509	1,599	1,832	1,900	1,949	1,954
Million pounds	3,326	3,526	4,040	4,190	4,297	4,308
<i>Fluid cream-light cream, heavy cream, half and half</i>						
1,000 MT	794	881	844	976	1,034	1,086
Million pounds	1,751	1,943	1,860	2,151	2,280	2,395
<i>Eggnog</i>						
1,000 MT	42	48	58	61	58	59
Million pounds	93	105	127	134	129	130
<i>Refrigerated yogurt</i>						
1,000 MT	833	909	1,048	1,137	1,228	1,356
Million pounds	1,837	2,003	2,310	2,507	2,707	2,990
<i>Sour cream and dips</i>						
1,000 MT	414	449	468	524	561	595
Million pounds	914	990	1,031	1,156	1,236	1,311
<i>Buttermilk</i>						
1,000 MT	282	269	261	248	239	232
Million pounds	622	592	576	547	527	512
<i>Butter</i>						
1,000 MT	570	559	615	563	566	611
Million pound	1,256	1,232	1,355	1,242	1,247	1,347
<i>Natural cheese</i>						
1,000 MT	3,746	3,747	3,877	3,881	4,025	4,140
Million pounds	8,258	8,260	8,547	8,557	8,873	9,127
<i>Processed cheese, foods, spreads, and cold pack</i>						
1,000 MT	1,038	1,001	1,056	1,091	1,016	1,030
Million pounds	2,288	2,207	2,327	2,406	2,240	2,270
Product	2000	2001	2002	2003	2004	2005
<i>Cottage cheese</i>						
1,000 MT	333	336	340	356	347	350
Million pounds	734	741	749	785	764	771
<i>All frozen desserts</i>						
Million liters	6,076.7	5,939.9	5,929.3	6,062.4	5,750.1	5,804.9
Million U.S. gallons	1,607.6	1,571.4	1,568.6	1,603.8	1,521.2	1,535.7
<i>Ice cream: regular</i>						
Million liters	3,702.9	3,666.9	3,798.9	3,753.2	3,515.0	3,602.3
Million U.S. gallons	979.6	970.1	1,005.0	992.9	929.9	953.0

Table 2.11. (cont.)

Product	Sales Volume					
	Year					
	2000	2001	2002	2003	2004	2005
<i>Low-fat and nonfat</i>						
Million liters	1,527.5	1,521.8	1,359.2	1,582.3	1,549.0	1,454.2
<i>Million U.S. gallons</i>	<i>404.1</i>	<i>402.6</i>	<i>359.6</i>	<i>418.6</i>	<i>409.8</i>	<i>384.7</i>
<i>Frozen yogurt</i>						
Million liters	357.2	198.8	276.6	266.8	243.8	246.1
<i>Million U.S. gallons</i>	<i>94.5</i>	<i>52.6</i>	<i>70.8</i>	<i>70.4</i>	<i>64.5</i>	<i>65.1</i>
<i>Sherbet</i>						
Million liters	196.2	243.4	215.5	204.5	207.5	223.4
<i>Million U.S. gallons</i>	<i>51.9</i>	<i>64.4</i>	<i>57.0</i>	<i>54.1</i>	<i>54.9</i>	<i>59.1</i>
<i>Water ices^a</i>						
Million liters	248.7	269.1	255.5	229.1	241.9	249.8
<i>Million U.S. gallons</i>	<i>65.8</i>	<i>71.2</i>	<i>67.6</i>	<i>60.6</i>	<i>64.0</i>	<i>66.1</i>
<i>Other frozen dairy products</i>						
Million liters	43.8	39.7	32.9	27.2	30.6	29.1
<i>Million U.S. gallons</i>	<i>11.6</i>	<i>10.5</i>	<i>8.7</i>	<i>7.2</i>	<i>8.1</i>	<i>7.7</i>

^aIncludes sorbet, frozen juice bars, gelatin pops.

MT, metric tons.

Source: Dairy Facts, 2006 edition. International Dairy Foods Association, Washington, DC.

in the world. Advanced processing and packaging technologies insure efficient delivery and shelf life of high-quality milk products (Chandan, 1997).

In 1982, there were 1,190 fluid milk-processing plants in the United States. As a result of consolidation, their number was reduced to 524 in 2002. During this period, the average volume of fluid milk processed per plant was more than double from 0.02 million MT (43.5 million pounds) to 0.05 million MT (105.5 million pounds). The total value of fluid milk and related products amounted to \$24 billion. The plants employed 55,400 workers receiving total compensation exceeding \$2.7 billion. More recently, the trend for further consolidation and increase in milk output per plant has continued.

In 2005, 53,036 dairy farms shipped 52 million MT (115 billion pounds) of milk to 302 dairy plants. At the plants, the farm milk is processed into an array of flavored and white milk ranging from fat-free to full fat fluid milk as well as half and half, coffee cream, and whipping cream as well as cultured products like sour cream and dips, and buttermilk. Other dairy plants manufacture more than 300 varieties of cheeses, hundreds of flavors of ice creams and frozen yogurt, and over 75 flavors of several types of refrigerated yogurt and yogurt drinks. Dairy plants also produce butter, sweetened condensed milk, evaporated

milk, dry milk, lactose, and whey products. More recently, the industry has demonstrated packaging and marketing innovations to compete aggressively for consumer food dollar share.

TRENDS IN THE SALES OF MILK PRODUCTS

In the year 2007, the products sold and their volumes are shown in Table 2.11.

Fluid Milk and Related Sales

Total sales of fluid milk products declined slightly from 2000 to 2005. The trend is shifting away from whole milk toward low- and nonfat milks. The volume of whole white milk sales in the United States dropped from 8.368 million MT (18,448 million pounds) in 2000 to 7.612 million MT (16,781 million pounds) in 2005. On the other hand, the sales of reduced and low-fat white milk have stayed fairly level from 10.847 million MT (23,913 million pounds) in 2000 to 10.71 million MT (23,611 million pounds) in 2005. Nonfat white milk sales decreased from 3.826 million MT (8,435 million pounds) in 2000 to 3.626 million tons (7,994 million pounds) in 2005. During the same time period, the sales of flavored milks

and drinks registered an increase from 1.509 million MT (3,336 million pounds) in 2000 to 1.086 million MT (4,308 million pounds) in 2005. Another trend is the significant growth in sales of organic milk and milk products. The sales of school milk jumped 6% in 2005 as compared to 2004. Buttermilk sales have declined while eggnog sales have increased.

Fluid cream sales have been increasing from 0.794 million MT (1,751 million pounds) in 2000 to 1.954 million MT (2,395 million pounds) in 2005. During this period, sour cream and dips increased to record levels. Yogurt sales have been spectacular, increasing from 0.833 million MT (1,837 million pounds) in 2000 to 1.356 million MT (2,990 million pounds) in 2005. Even at present, the sales are showing remarkable growth. Buttermilk sales are declining every year.

Butter

The sales grew from 0.57 million MT (1,256 million pounds) to 0.611 million MT (1,347 million pounds) from 2000 to 2005.

Cheese

There are 300 varieties of cheese available in the United States. Cheddar and Mozzarella cheese are the most popular cheeses. American and Italian cheeses account for 83% of all cheese sales. The sales of natural cheese continued to climb from 3.746 million MT (8,258 million pounds) in 2002 to 4.14 million MT (9,127 million pounds) in 2005. During the same period, the sales of processed cheese, spreads, and cold pack were fairly steady, while Cottage cheese sales have not shown much growth in the recent years.

Ice Cream and Frozen Desserts

Total retail value of the ice cream industry in 2004 was \$20.6 billion, which increased to \$21.6 billion in 2005. Ice cream comprises 87% of the total frozen dessert volume. The rest of the market is ascribed to sherbet, frozen yogurt, and water ices. The ice cream category consists of regular and low-fat plus nonfat ice cream, each of which is further divided into hard and soft varieties.

The sales of regular ice cream have declined from 3,702.9 million liters (979.6 million gallons) to 3,602.3 million liters (953 million gallons) from 2000 to 2005. During the same period, sales of low-fat and nonfat ice cream also decreased from 1,527.5 million

liters (404.1 million gallons) to 1,454.2 million liters (384.4 million gallons). Frozen yogurt declined appreciably, while sherbet and water ices declined slightly. In general, total sales of all frozen dairy products declined from 6,076.7 million liters (1,607.6 million gallons) in the year 2000 to 5,804.9 million liters (1,535.7 million gallons) in 2005.

Production of Industrial and Other Dairy Products

Table 2.12 shows the production data for certain dairy products including several ingredients used in dairy and food product manufacture.

To meet the demand of manufacturing industry, nonfat dry milk production has been fairly steady in recent years, recording 6.84 million MT (1,509 million pounds) in 2005. Evaporated and condensed whole milk has increased from 0.2 million MT (442 million pounds) in 2000 to 0.239 million MT (527 million pounds) in 2005. During the same period, evaporated skim milk declined somewhat and was 0.009 million MT (20 million pounds) in 2005. Bulk condensed whole milk increased appreciably from 0.066 million MT (146 million pounds) in 2000 to 0.082 million MT (179 million pounds) in 2005. During the same period, bulk condensed skim production stayed steady at 0.479 million MT (1,057 million pounds) in 2005. Condensed buttermilk registered an increase from 0.009 million MT (20 million pounds) in 2000 to 0.035 million MT (78 million pounds) in 2005.

The production of total whey products was 0.976 million MT (2,151 million pounds) in 2000 and 0.98 million MT (2,161 million pounds) in 2005. Dry whey production was 0.474 million MT (1,046 million pounds) in 2005, remaining steady in the previous years, whereas condensed sweet whey production decreased to 0.036 million MT (79.2 million pounds) in 2005. Reduced lactose and mineral whey products declined sharply from 0.052 million MT (114 million pounds) in the year 2000 to 0.02 million MT (44 million pounds) in 2005. Whey protein concentrate production flourished and it registered 0.147 million MT (324 million pounds) in 2005. So was lactose production, increasing from 0.222 million MT (490 million pounds) in the year 2000 to 0.303 million MT (668 million pounds) in 2005.

PER CAPITA CONSUMPTION

The data for major consumer dairy foods from the year 2000 to 2005 are shown in Table 2.13.

Table 2.12. Production of Industrial and Other Dairy Products in the United States During the Years 2000–2005

Product	Production Volume (Millions of Pounds) in the Year					
	2000	2001	2002	2003	2004 ^r	2005 ^p
Nonfat dry milk						
1,000 MT	659	641	724	721	640	684
<i>Million pounds</i>	1,452	1,414	1,596	1,589	1,412	1,509
Evaporated and condensed whole milk						
1,000 MT	200	205	260	262	240	239
<i>Million pounds</i>	442	453	573	578	530	527
Evaporated skim milk						
1,000 MT	11	7	9	8	9	9
<i>Million pounds</i>	24	15	20	17	19	20
Bulk condensed whole milk						
1,000 MT	66	63	60	93	88	82
<i>Million pounds</i>	146	140	133	204	193	179
Bulk condensed skim milk						
1,000 MT	479	440	480	427	424	479
<i>Million pounds</i>	1,057	970	1,058	942	935	1,057
Condensed evaporated buttermilk						
1,000 MT	9	16	25	19	23	35
<i>Million pounds</i>	20	35	56	41	50	78
Dry whey						
1,000 MT	501	444	506	492	469	474
<i>Million pounds</i>	1,105	979	1,115	1,085	1,035	1,046
Condensed sweet whey—human grade						
1,000 MT	52	37	49	52	41	36
<i>Million pounds</i>	115	81	108	115	91	79
Reduced lactose and minerals						
1,000 MT	52	59	57	19	18	20
<i>Million pounds</i>	114	129	125	43	40	44
Whey protein concentrate						
1,000 MT	132	132	124	139	135	147
<i>Million pounds</i>	290	290	274	306	298	324
Lactose						
1,000 MT	222	235	255	278	302	303
<i>Million pounds</i>	490	519	563	614	665	668
Total whey products						
1,000 MT	976	925	1,008	981	966	980
<i>Million pounds</i>	2,151	2,039	2,223	2,163	2,130	2,161

MT, metric tons; r, revised; p, preliminary.

Source: Dairy Facts, 2006 edition. International Dairy Foods Association, Washington, DC.

Fluid Milk and Related Products

As shown in Table 2.13, the 2005 total per capita sales of all fluid milk products in the United States were 74.5 kg (181 pounds) or approximately 79 liters (21 gallons). In general, the per capita consumption of unfavored whole milk, reduced fat milk, low-fat milk and nonfat milk, and buttermilk is going down, while the consumption of flavored milk and fluid

cream is going up. Flavored milks have shown good growth and helped to balance partially the decline in per capita consumption of white milk beverages. Sour cream and dips, and yogurt achieved record sales in 2005. Yogurt in particular has consistently shown remarkable per capita consumption in the last decade, registering a record per capita consumption of 3.9 kg (8.6 pounds) in 2005.

Table 2.13. Per Capita Consumption of Retail Dairy Products in the United States

Product	2000		2001		2002		2003		2004		2005 ^p	
	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds
Whole white milk	22.9	66.1	28.9	63.8	28.5	62.9	28.1	61.9	26.8	59.2	25.7	56.6
Reduced fat white milk	27.8	61.3	27.5	60.6	27.2	60.1	27.1	59.7	26.9	59.3	26.8	59.2
Low-fat white milk	10.2	22.5	10.1	22.3	9.9	21.8	9.7	21.3	9.6	21.1	9.7	21.5
Nonfat white milk	13.6	29.9	13.1	28.9	12.7	27.9	12.2	26.8	12.0	26.5	12.3	27.0
Flavored milk drinks	5.4	11.8	5.6	12.4	6.4	14.0	6.5	14.4	6.5	14.6	6.6	14.5
Buttermilk	1.0	2.2	0.9	2.1	0.9	2.0	0.9	1.9	0.8	1.8	0.8	1.7
Eggnog	0.1	0.3	0.2	0.4	0.2	0.4	0.2	0.5	0.2	0.4	0.2	0.4
Fluid cream	2.8	6.2	3.1	6.8	2.9	6.5	3.4	7.4	3.6	7.9	3.7	8.1
Sour cream	1.4	3.2	1.6	3.5	1.6	3.6	1.8	4.0	1.9	4.2	2.0	4.4
Yogurt	2.9	6.5	3.2	7.0	3.4	7.4	3.7	8.2	4.2	9.2	3.9	8.6
Cottage cheese	1.2	2.6	1.2	2.6	1.2	2.6	1.2	2.7	1.2	2.7	1.2	2.6
Cream and Neufchatel cheese	1.1	2.4	1.0	2.3	1.1	2.4	1.1	2.3	1.1	2.4	1.1	2.3
Natural cheeses	13.5	29.8	13.6	30.0	13.8	30.5	13.8	30.5	14.1	31.2	14.2	31.4
Process cheese, food, and spread	3.7	8.1	3.5	7.7	3.7	8.1	3.7	8.2	3.5	7.7	3.4	7.6
Regular ice cream—hard ^a	11.8	12.5	11.5	12.2	11.4	12.1	11.4	12.0	10.9	11.5	11.3	11.9
Regular ice cream—soft ^a	1.3	1.4	1.3	1.4	1.7	1.8	1.5	1.6	0.9	1.0	0.8	0.9
Low-fat and nonfat ice cream—hard ^a	1.5	1.6	1.4	1.5	1.6	1.7	1.5	1.6	1.5	1.6	1.4	1.5
Low-fat and nonfat ice cream—soft ^a	3.8	4.0	3.9	4.1	3.1	3.3	3.8	4.1	3.6	3.8	3.2	3.4
Sherbet—hard ^a	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Sherbet—soft ^a	0.04	0.04	0.04	0.04	0.05	0.05	0.02	0.02	0.05	0.05	0.05	0.05
Other frozen desserts—hard ^a	1.6	1.7	1.5	1.5	1.5	1.5	1.3	1.4	1.3	1.4	1.3	1.4
Other frozen desserts—soft ^a	0.7	0.7	0.4	0.5	0.4	0.5	0.5	0.6	0.4	0.5	0.4	0.5
Total frozen desserts—hard ^a	15.6	16.5	15.1	16.0	15.2	16.1	14.9	15.8	14.3	15.2	14.7	15.5
Total frozen desserts—soft ^a	5.8	6.2	5.7	6.0	5.3	5.6	5.9	6.3	5.0	5.3	4.5	4.8

^a In liters, followed by U.S. quarts.

p, preliminary.

Source: Dairy Facts, 2006 edition. International Dairy Foods Association, Washington, DC.

Table 2.14. Per Capita Sales of Selected Manufactured Dairy Products in the United States

Dairy product	2000		2001		2002		2003		2004 ^r		2005 ^p	
	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds	kg	Pounds
Butter	2.0	4.5	2.0	4.4	2.0	4.4	2.0	4.5	2.1	4.6	2.1	4.6
Nonfat dry milk	2.0	2.6	1.4	3.2	1.4	3.1	1.5	3.4	1.9	4.3	1.4	3.0
Dry whole milk	0.1	0.3	0.1	0.2	0.1	0.2	0.05	0.1	0.05	0.1	0.05	0.1
Dry whey	1.7	3.8	1.5	3.4	1.5	3.4	1.5	3.4	1.4	3.2	1.3	2.9
Evaporated and condensed whole milk	0.9	2.0	0.9	2.0	1.0	2.3	1.2	2.6	1.3	2.9	1.0	2.1
Evaporated and condensed skim milk	1.7	3.8	1.5	3.4	1.7	3.7	1.5	3.3	1.5	3.2	1.6	3.6

r, revised; p, preliminary.

Source: Dairy Facts, 2006 edition. International Dairy Foods Association, Washington, DC.

Cheese

The per capita consumption of natural cheese reached record levels of 14.2 kg (31.4 pounds) in 2005, reflecting its continuous popularity. American and Italian cheeses account for 83% of all cheese sales. During the same year, Cheddar cheese accounts for 4.59 kg (10.11 pounds) per capita, while Mozzarella cheese is 4.63 kg (10.18 pounds) per capita. The consumption of American-type cheeses fell by 1.12% to 5.77 kg (12.7 pounds). The per capita consumption of Italian-type cheeses increased by 3.2% to 6.04 kg (13.3 pounds), largely due to consumption growth in Mozzarella cheese. Other natural cheeses showed per capita sales of 2.45 kg (5.4 pounds).

The per capita consumption of Cream and Neufchatel, Muenster, Blue, and Brick cheeses in 2005 was 1.06 kg (2.33 pounds), 0.12 kg (0.26 pound), and 0.09 kg (0.20 pounds), respectively, and their trend was fairly steady from 2000 to 2005. Swiss cheese consumption is growing slightly and it was 0.56 kg (1.24 pounds) in 2005. Hispanic cheeses have grown from 0.15 kg (0.34 pound) per capita to 0.25 kg (0.56 pound) per capita.

The per capita consumption of processed cheese has been reduced from 2.2 kg (4.85 in 2000) to 1.87 kg (4.13 pounds) in 2005. During the same period, the per capita consumption of processed cheese and spreads increased from 1.45 kg (3.19 pounds) to 1.58 kg (3.47 pounds). Overall, the per capita consumption of total processed cheeses registered a decrease from 3.7 kg (8.1 pounds) to 3.4 kg (7.60 pounds).

Ice Cream and Frozen Desserts

The total per capita consumption of all frozen desserts has declined from 21.46 liters (22.71 quarts) in 2000 to 19.21 liters (20.33 quarts) in 2005.

Regular ice cream comprises hard and soft varieties that altogether represent 63% of the total ice cream market. The rest of the market is consisted of hard and soft low-fat and nonfat ice creams. The per capita consumption of regular hard ice cream declined from 11.8 liters (12.5 quarts) in 2000 to 11.3 liters (11.9 quarts) in 2005, while the soft counterpart declined from 1.3 liters (1.38 quarts) to 0.8 liter (0.86 quarts). In case of low-fat and nonfat ice creams, the per capita consumption of hard variety declined from 1.5 liters (1.62 quarts) to 1.4 liters (1.47 quarts) and

the soft variety decreased from 3.8 liters (4.04 quarts) to 3.2 liters (3.42 quarts).

Per Capita Consumption of Industrial and Other Products

Table 2.14 shows the trend in the consumption of dairy products generally sold for use in manufacturing other dairy foods and for making other food products.

In general, per capita butter consumption has slightly increased from 2 kg (4.5 pounds) in 2000 to 2.1 kg (4.6 pounds) in 2005.

Nonfat dry milk consumption in 2005 was 1.4 kg (4.6 pounds) and has remained fairly constant since 2000. Dry whole milk consumption on per capita basis has declined from 0.1 kg (0.3 pound) in 2000 to 0.05 kg (0.1 pound) in 2005. Dry whey consumption has declined from 1.7 kg (3.8 pounds) in 2000 to 1.3 kg (2.9 pounds) in 2005.

The per capita consumption of evaporated and condensed whole milk was 1 kg (2.1 pounds) in 2005, while the skim milk product consumption was 1.6 kg (3.6 pounds). Both the products have not shown any definite trend in per capita consumption.

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3

Mammary Gland and Milk Biosynthesis: Nature's Virtual Bioprocessing Factory

Ramesh C. Chandan, Dilip A. Patel, Raul A. Almeida, and Stephen P. Oliver

Introduction
Mammary Gland
Biosynthesis of Milk Proteins
Milk Enzymes
Biosynthesis of Milk Lipids
Biosynthesis of Milk Sugar, Lactose
Secretion of Milk Constituents Into Lumen
Milk FGM
Rate of Milk Secretion
Current and Future Mammary Gland-Based Research
Compositional Quality
Healthy Milk Fat
Genetic Polymorphism and Enhancing Cheese Quality and Yield
Bioactive Peptides
Fighting Intramammary Infections
Conclusions
References

INTRODUCTION

Domestication of animals and milk production is estimated to have begun approximately 6,000 years ago. Domestication of animals was probably motivated by dire need for milk, meat, and clothing, and so forth. Since herbivorous animals were easier and safer to domesticate and posed little threat to humans, they became the animals of choice. Such animals could also thrive on agricultural byproducts and waste products. Cows are the most common dairy animal in the world. However, water buffalo, goat, sheep, camel, and mare also contribute significantly to milk production in certain parts of the world.

Milk is an excellent functional and live biological fluid (Chandan, 2007b). It is secreted by about 4,000 species of mammals for providing complete

nutritional needs of their offspring. The young of the species depends on the mother's milk not only for growth and development, but also for vital immune support during early stages of life. Vital protective factors such as immunoglobulins and antibacterial factors such as lysozyme and lactoferrin in mother's milk provide much protection against most childhood infections. Furthermore, milk supplies a myriad of constituents to help in digestion processes (proteinases, lipases), and hormones essential for growth of the neonate (Fox, 2003). Growth supporting (through essential growth hormones) and digestion enhancing (through various proteinases and lipases) properties of milk are vital for growth and development.

The nutritional and physiological requirements of different species are different. Consequently, milk composition is species-specific and therefore, varies considerably. Table 3.1 shows the composition of milk of various species to illustrate this point. More details on milk composition are given in another publication (Chandan, 2007a) and in Chapter 4 of this book.

Colostrum is the milk from first and subsequent 72-hour of milking of cow immediately after calving. Its composition is markedly different from normal milk. It contains high amounts of milk solids as well as copious quantities of immunoglobulins to transfer immunity from mother to the offspring. Commercial milk in the United States is not permitted to contain colostrum.

Being animal-derived biological fluid, milk is a live system that undergoes dynamic physicochemical and biochemical changes as a result of enzymatic, physical, or chemical abuses, insults, injuries, or repairs/rescue encountered in the entire food

Table 3.1. Proximate Composition of Milk of Various Species

Mammal	% Total Solids	% Protein	% Lipids	% Lactose	% Ash
Woman	12.2	1.2	3.8	7.0	0.2
Cow	12.6	3.4	3.7	4.8	0.7
Buffalo	16.8	3.8	7.4	4.8	0.8
Goat	13.2	3.6	4.1	4.7	0.8
Sheep	18.7	5.5	7.4	4.8	1.0
Mare	11.1	2.5	1.9	6.2	0.5
Donkey	11.3	2.0	1.4	7.4	0.5
Sow	16.4	4.9	5.3	5.3	0.9
Camel	13.4	3.6	4.0	5.0	0.8
Rabbit	34.1	11.9	18.3	2.1	1.8
Reindeer	33.1	11.5	16.9	2.8	NA
Elephant	21.9	4.9	11.6	4.7	0.7
Polar bear	45.7	10.9	33.1	0.3	1.4
Grey seal	65.7	11.2	53.1	0.7	NA
Dog	20.4	7.1	8.3	3.7	1.3

NA, not available.

Sources: Fox (2003), Stelwagen (2003a), Jensen (1995), and Campbell and Marshall (1975).

chain. Milk composition continues to change after milking, depending on how it is stored or treated. Physical and chemical equilibria related to proteins and minerals are vulnerable to shifts caused by changes in pH and temperature. The enzymes of raw milk could induce alterations in oxidation/reduction system, and degradation of proteins and lipids. Similarly, the growth of microorganisms could induce a cascade of changes. Loss of CO₂ from raw milk changes acid–base equilibrium. The structure of milk fat globule membrane (FGM) is in flu and vulnerable to changes. Indeed, manipulation of chemical system (chemical structure and associated function), enzyme system, and resultant physical structure holds tremendous promise for optimizing processing, technological, nutritional, and therapeutic aspects of milk and milk-derived foods. It is now recognized that barring microbiological and toxicants associated with food safety and quality issues, chemical system consisting of enzymes and associated chemistry, and milk biomolecules and associated structure function chemistry holds very good promise to enhance technological, processing, and nutritional outcomes in food processing. It is no wonder then that mammary gland, where intense biochemical activity and bioprocessing occurs, has become the focus of modern dairy research. Veteran dairy chemist Stuart Patton compared mammary gland with biological factory and rightly predicted that mammary epithelial cells would rank second only to photosynthesis cells in sustaining mammalian life (Patton, 1969). Accord-

ing to Bauman et al. (2006), the productivity of mammary gland is extensive and in terms of the use of nutrients and energy, the cow should be considered an appendage to the mammary gland and not vice versa.

MAMMARY GLAND

Milk synthesis occurs in the mammary gland (consisted of specialized mammary epithelial cells) situated in the udder of cow (see Figures 3.1 and 3.2).

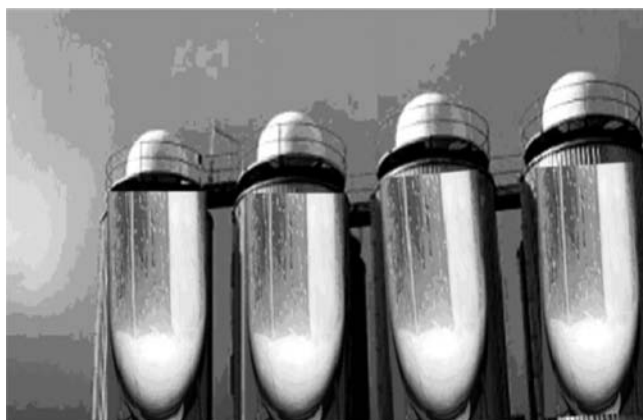
Anatomically, cow udder is semicircular with two halves, divided by a crease. Each half in turn, is divided into two individual quarters separated by a shallower crease constituting total four quarters (also called four chambers). Each quarter is independent of each other and is equipped with its own mammary gland that is connected to one teat end meant for milk withdrawal. Figure 3.3 shows mammary epithelial cells.

The figure also shows the cells of a pathogenic organism adhering to epithelial cells. Mastitis is caused by the invasion of several types of organisms, resulting in impaired milk quality. In general, the severity of mastitis is indicated by a high somatic cell count in milk. High somatic cell count is likely to affect the keeping quality of pasteurized and ultra-high temperature (UHT) milk (Boland, 2003).

The udder of cow is protected by an outer layer of muscular tissue that accords cohesion to the udder body and protects it from external hits or abrasion. Inside, the glandular tissue is consisted of a



(a)



(b)

Figure 3.1. An artistic view of mammary gland as a bioprocessor.

large number of alveoli, which act like tiny bladders. Milk-secreting cells are located in the inner walls of the alveoli. Secreted milk is carried by capillaries into milk ducts, which lead into cavity known as cistern, located above the teat. Cistern holding about one third of milk of the udder, is connected to teat. The teat contains a channel that allows milk to exit from teat. A sphincter muscle is located at end of the teat channel to close the fl w of milk between milking. The muscle prevents milk from leaking out of the teat and controls entry of bacteria and other harmful microbes. During hand or machine milking, the hormone oxytocin allows “let down” of milk and initiates emptying of milk from udder. Preparation of cow for milking includes stimulation of udder by calf suckling or by other stimuli in the milking parlor signals release of the hormone into blood. The “let down” of milk by oxytocin is complete in less than a

minute. The muscle-like cells of alveoli develop pressure in the udder, forcing milk into teat cistern from where it can be withdrawn by suction of milking machine or by applying pressure on the teat during hand milking. The effect of oxytocin lasts for 5–8 minutes, during which milking process should be completed.

Mammary gland has a sophisticated level of organization with a remarkable ability to convert circulating nutrients from blood into milk components (Bauman et al., 2006). Mammary epithelial cells in mammary gland synthesize complex milk constituents from simple components present in circulating blood. Mammary gland is equipped with an extensive network of arterial and venous blood capillaries. The components of blood needed for milk biosynthesis are extracted from arterial blood. The venous blood carries the blood back to the heart for recirculation and component renewal. On the basis of

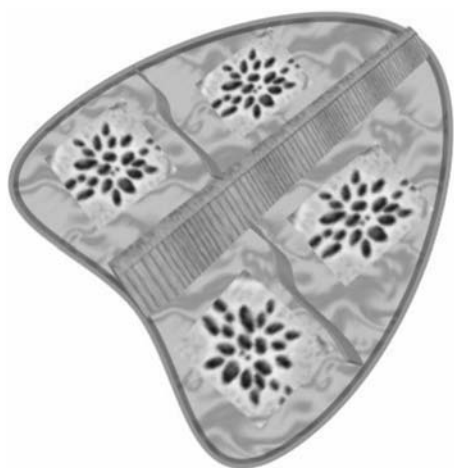


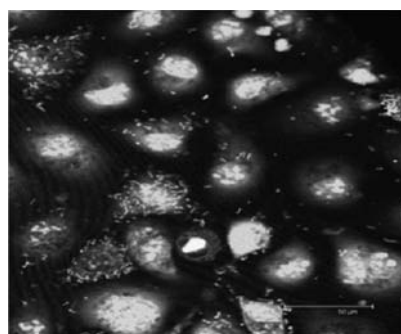
Figure 3.2. Schematic of cow udder showing four quarters (chambers) each separated by muscular tissue.

the percentage of precursor difference in arterial and venous blood, the ability of mammary gland to extract milk precursors from arterial blood is astounding in that it could approach as high as 20 L/min (Weimer, 2001).

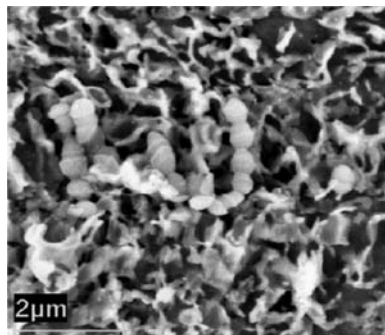
Precursors of milk proteins, lipids, and carbohydrates are present in blood. All the milk fat, lactose, and most milk proteins are then synthesized by the mammary epithelial cells. Certain milk constituents (milk fat, most milk proteins) are specific to species of the animal. Lactose is also synthesized in the mammary gland but it is identical in all species of mammals. Water, salts, vitamins, immunoglobulins, some hormones, and serum albumin leak into milk unchanged from blood. It is generally agreed that to synthesize 1 liter of milk, the epithelial cells of mammary gland require 800–900 liters of blood (Bylund, 1995) moving through them for providing adequate level of milk precursors. Lactating mammary gland extracts glucose, amino acids, fatty acids, β -hydroxy butyrate, and mineral salts from blood for synthesis of milk constituents. The epithelial cells synthesize milk continuously from the precursors and store milk until it is removed.

The composition of blood and milk is quite different (Table 3.2).

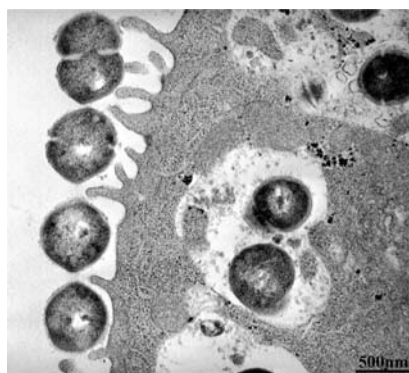
The ratios of major milk and blood components suggest that fat, sugar, potassium, calcium, magnesium, and phosphate are several times more abundant in milk than in blood. However, the concentration of



(a)



(b)



(c)

Figure 3.3. Confocal laser (a), scanning electron (b), and transmission electron (c) micrographs of mammary epithelial cells. Frequently, bovine udder gets infected with mastitis pathogens affecting milk quality. *Streptococcus uberis* cells are shown adhering and invading cultured mammary epithelial cells.

protein, phospholipid, sodium, and chloride is significantly more in blood than in milk.

The pH of milk is 6.6, which equals H^+ concentration of 2.51×10^{-7} . The pH of arterial and venous blood is 7.45 and 7.35, respectively. This corresponds to hydrogen ion concentration of 3.55×10^{-8} in

Table 3.2. Comparative Proximate Composition of Blood and Milk of Cow

Major Constituent	Concentration (g/L in Milk)	Concentration (g/L in Blood)
Water	860	910
Triacylglycerols	37	0.6
Phospholipids	0.35	2.5
Amino acids, free	Trace	0.2
Caseins	28	0
β -Lactoglobulin	3.2	0
α -Lactalbumin	1.3	0
Serum albumin	0.5	32
Immunoglobulins	0.7	26
Glucose	Trace	0.5
Lactose	46	0
Citric acid	1.8	Trace
Orotic acid	0.008	0
Calcium	1.3	0.1
Phosphorus	1.0	0.1
Sodium	0.5	3.4
Potassium	1.5	0.25
Chloride	1.1	3.5

Adapted from Larson (1985).

arterial blood and 4.47×10^{-8} in venous blood. Thus, the hydrogen ion concentration in milk is 5.6–7.1 times higher in milk as compared to blood. As per Donnan equilibrium, the difference in H^+ ions concentration represents a potential of $0.00019832 (273 + 38) \log_{10} 5.6/1 = 0.046$ volt. This difference exists between blood and milk. That difference may explain the uneven distribution of solutes in secretory processes. For low dissociation constant materials (like urea, creatine, creatinine) an even distribution is expected. Lactose is not dialyzed into blood apparently because of equilibrium with respect to osmotic pressure even under the condition of secretion. Since milk and blood have same osmotic pressure, they are isotonic. In two fluid separated by semipermeable membrane, pressure required for stopping osmosis or migration of diffusible solutes across the membrane equals osmotic pressure.

According to Donnan effect, dialyzable ions exert influence on osmotic pressure. Let us assume the concentration of Na^+ ion both inside and outside the membrane is C_1 and the concentration of Cl^- is C_2 . The concentration of nondiffusible particles like protein, oleate, is R^- inside the membrane. After equilibrium is attained, some concentration, say, x of Cl^- ions will diffuse across the membrane, raising its

concentration to $C_1 + x$. The concentration of Cl^- outside the membrane will be reduced to $C_2 - x$. Concomitantly, as Cl^- diffuses into the membrane, an equivalent amount of Na^+ also diffuses out simultaneously to maintain neutrality of charge. Thus, ion concentration of Na^+ increases to $C_1 + x$ inside the membrane, while its concentration is reduced to $C_1 - x$ outside the membrane. The product of diffusible ion concentration is equal.

$$x(C_1 + x) = (C_2 - x)^2$$

$$\text{Solving for } x \text{ gives } x = \frac{C_2^2}{C_1 + 2C_2}$$

Example: Let $C_1 = 0.01$ molar
 $C_2 = 1.00$ molar

$$x = \frac{C_2^2}{C_1 + 2C_2} = \frac{1}{2.01} = 0.497$$

Thus, concentrations inside the membrane are:

$$Na^+ = 0.497 + 0.01 = 0.507$$

$$R^- = 0.01$$

$$Cl^- = 0.497$$

$$\text{Total} = 1.014$$

Concentrations outside the membrane are:

$$Na^+ = 1.00 - 0.497 = 0.503$$

$$Cl^- = 0.503$$

$$\text{Total} = 1.006$$

Difference in concentration between inside and outside membrane equals $1.014 - 1.006 = 0.008$

$$\text{Osmotic pressure } P = \frac{nRT}{V},$$

where n is the difference in concentration between inside and outside membrane, R is a constant = 0.082 liter/atmosphere/mole/degree, T is absolute temperature = $273 + 25 = 298^\circ K$, and V is volume = 1 liter.

Solving for P ,

$$P = \frac{0.008 \times 0.082 \times 298}{1} \text{ atmosphere} = 0.195.$$

This figure has been experimentally verified and found to be in agreement.

Major milk constituents, their physical state and particle size after biosynthesis in the mammary gland are shown in Table 3.3.

Fat and protein are in colloidal dispersion; fat as emulsified globules with membranous coating and

Table 3.3. Physical State and Particle Size Distribution in Milk

Compartment	Size, Diameter (nm)	Type of Particles
Emulsion	2,000–6,000	Fat globules
Colloidal dispersion	50–300	Casein–calcium phosphate
	4–6	Whey proteins
True solution	0.5	Lactose, salts, and other substances

Adapted from Chandan (2007a).

proteins as micelles. The minerals, vitamins, and lactose are in true solution form. The growth rate of an animal is inversely related to the nutrient density (total solids, fat level, calories) of the milk secreted by the mammal (Jensen, 1995). Both protein and minerals, particularly calcium and phosphorus, are building blocks during muscle and skeletal development. Human milk is relatively low in protein and ash (mineral) content in relation to cow's and dog's milk. It takes 180 days for human baby to double its weight, whereas it takes only 47 and 8 days for calf and puppy to double its birth weight respectively.

In case of modern dairy animals bred for milk production, this relationship does not hold because they are genetically selected and bred for high milk yield. In this context, milk yield of dairy cows, goats and water buffaloes surpasses the need of the offspring. On an average, modern cow is capable of producing as much as 9,400 kg of milk per lactation period, while milk requirement of a calf is around 1,000 kg. The surplus milk is therefore available for human consumption. It is recognized that milk of particular species of mammal is best suited for neonate of the same species immediately after birth. However, the milk of common milking animals can be adapted for interspecies consumption. For example, by altering the qualitative and quantitative composition of cow's milk in terms of fat, protein, and minerals, human infants can utilize the nutrients for growth and development. Similarly, economical calf milk replacers based on less expensive food ingredients fulfil adequate nutritional needs of growing calf, thereby enhancing availability of even more milk for human consumption.

Milk production begins soon after the cow has delivered calf. The heifer generally undergoes artificial or natural insemination around 15–18 months of age. The gestation period is 265–300 days, depending on the breed of the cow. Accordingly, a heifer calves for the first time at age of 2–2.5 years. Although milk production continues for about 10 months, the cow is bred again after 1–2 months of calving. During lac-

tation period, production of milk declines 15–20% from the peak after calving. Normal lactation period in cow is 305 days. At this point milking is stopped to allow nonlactation period of about 60 days before the birth of new calf. The process continues for 5–6 lactation periods (Bylund, 1995).

From human nutrition standpoint, milk is considered as nature's nearly perfect food. The role of bovine milk in human diet for its contribution of high-quality protein, amino acids, minerals, particularly calcium and fat has been recognized for many centuries. The reader is referred to Chapter 18 of this book for detailed discussion on nutritive role of milk in human nutrition. Lately, one of its constituents (milk fat) has received adverse publicity due to its high saturated fatty acid content. It is now recognized that it may be possible to improve the nutritional quality of milk by judicious selection of animal breeding and dietary feeding practices. For details, the reader is referred to the chapter by Givens and Shingfield (2003).

BIOSYNTHESIS OF MILK PROTEINS

For biosynthesis of milk constituents, the primary substrates extracted by mammary epithelial cells from their counterparts in blood include glucose, amino acids, fatty acids, β -hydroxy butyrate, and salts. Knowledge relative to biosynthesis of milk constituents is not fully understood; therefore, it is an evolving science.

In the ruminant animals, all food must pass through rumen prior to digestion in the stomach and intestines. A large proportion of dietary protein is transformed by rumen bacteria and protozoa, thereby generating high-quality microbial protein with significantly better amino acid profile than that of the vegetable protein in the feed. After digestion, the microbial protein along with smaller quantity of feed protein (that escaped rumen digestion) gives rise to small peptides and amino acids. These are then

Table 3.4. Approximate Concentration of Protein Fractions in Cow's Milk

Fraction	% in Skim Milk	% in Milk Protein	% Total Casein	% Total Whey Protein
Total protein	3.27	100.0	—	—
Total casein	2.69	82.2	100.0	—
α -s ₁ -Casein	1.02	31.3	38.1	—
α -s ₂ -Casein	0.27	8.4	10.2	—
β -Casein	0.96	29.3	35.7	—
κ -Casein	0.35	10.5	12.8	—
γ -Casein	0.09	2.7	3.2	—
Total whey protein	0.57	17.8	—	100.0
β -Lactoglobulin	3.14	9.6	—	54.2
α -Lactalbumin	1.23	3.8	—	16.8
Bovine serum albumin	0.45	1.4	—	7.8
Immunoglobulins, lactoferrin, proteose peptones fraction-3	0.97	3.0	—	21.2

Adapted from Stelwagen (2003a,b) and Chandan (2007a).

transported across the intestinal wall into blood, which ultimately form the substrate for protein synthesis in mammary gland.

The biosynthesis of milk proteins in mammary gland resembles biosynthesis of any other protein in body tissues. The substrate, amino acids from blood, is transported through the basolateral membrane to mammary secretory cell. The transporting systems may be sodium dependent or independent. Different groups of amino acids require different transporting system. Milk proteins are encoded by specific genes in the genome. The biosynthesis is initiated by gene expression which itself gets initiated by the hormone-induced transcription factors. Following essential steps are involved in protein biosynthesis:

1. *Transcription* occurs in the cell nucleus. It involves formation of messenger RNA, which carries the code of a specific protein. The mRNA is assembled in ribosomes attached to the rough endoplasmic reticulum (RER).
2. *Activation* of amino acids in the cytoplasm takes place by reaction with ATP and subsequent attachment to transfer RNA (tRNA). Each tRNA is specific for an amino acid.
3. *Translation* occurs in the ribosome. The code for amino acids resides in mRNA. Each code comprises three nucleotides called codon. A trinucleotide called anticodon is contained in the tRNA, which recognizes the codon. Each codon comes into position and appropriate amino acid-tRNA complex is added to form peptide chain.

Within the attached ribosomes of the RER, the protein polypeptide chain is synthesized with the addition of a chain of 10–20 amino acids at a time. In the next phase, the initial signal peptide sequence mediates the passage of the initial peptide chain through the membrane to the inner passages and gets clipped off. The polypeptide chain then folds up in a configuration dictated by the physical forces inherent to the sequence of the amino acids. Other groups like phosphates of calcium as in case of casein are added later. Finally, the protein assumes its three-dimensional structure that gives the protein its distinctive function. Following synthesis, milk proteins being secretory proteins are transported from the cell into alveolar lumen to merge with other milk constituent pool.

The proximate composition of major milk proteins is shown in Table 3.4.

Total nitrogen distributed among various fractions is caseins (76%), whey proteins (18%), and nonprotein nitrogen (6%). In order to assay true proteins, nonprotein nitrogen content is subtracted from total nitrogen content. Basically, true milk proteins are classified as caseins and whey (or serum) proteins. The classification is based on solubility at pH 4.6, at which caseins become insoluble, whereas whey proteins remain soluble. From the standpoint of physical structure, casein exists as calcium phosphate complex in the form of colloidal suspension, while whey proteins occur in soluble form. Another type of milk protein occurs as a part of milk FGM, covering the envelope in which milk fat is enclosed. Casein molecules in milk occur as spherical particles called micelles.

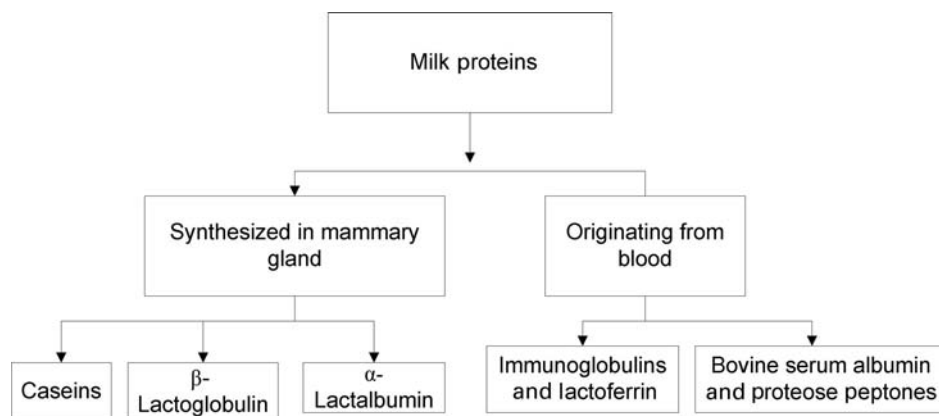


Figure 3.4. Origin of milk proteins.

Micelles are composed of several thousands of calcium phosphate–casein complexes.

Caseins are further subdivided into distinct fractions, α -s₁-casein, α -s₂-casein, β -casein, and κ -casein. These caseins are expressed by different genes and depend on the cow's different genetic variants. Another fraction, γ -casein is observed, but it is known to be a degradation product of β -casein catalyzed by proteolytic enzyme, plasmin.

Proteins originating from mammary gland synthesis are shown in Figure 3.4.

The milk proteins originating directly from blood are also shown. Immunoglobulins and bovine serum albumin, lactoferrin, and proteose peptones belong to this later category. They enter mammary gland via plasma cell adjacent to the secretory epithelium and are spilled into milk unchanged. Milk also contains various enzymes derived from biosynthetic activity. Urea, creatine, and creatinine are nonprotein nitrogen compounds, which originate from blood as well. Minerals of milk are derived from blood and their level is determined by Donnan equilibrium and osmotic conditions. Calcium, phosphate, and citrate form stable complexes with caseins to create micelles which are then secreted into milk.

Milk proteins are unique and exclusive class of proteins in that they contain more amino acids as compared to other food proteins. Milk proteins are also very valuable in that they not only supply essential and nonessential amino acids, but also generate bioactive peptides with strong biological activity. For example, specific bioactive peptides derived from caseins and whey proteins exhibit attributes of provid-

ing opioid agonistic activity, increasing gut motility, promoting cell growth and repair, reducing hypertension, preventing cancer, and stimulating and regulating immune system. For more details, see Chapter 18 of this book.

Some milk proteins have been shown to possess intracellular function. In this regard, α -lactalbumin is known to be associated with lactose synthase in lactose biosynthesis in the mammary gland. Calcium and phosphate associated with caseins promote bone growth. The micelles of casein are highly digestible providing calcium and phosphorus for skeletal development. The phosphopeptides originating from caseins help in utilization of minerals, namely iron, manganese, selenium, and calcium by sequestering them in soluble complexes for easy absorption.

MILK ENZYMES

Milk is a repository of myriad of enzymes. Over 60 indigenous enzymes have been reported in cow's milk. They are either associated with milk FGM (xanthine oxidase, sulfhydryl oxidase, and γ -glutamyltransferase), or with skim milk fraction (catalase, superoxide dismutase), or with micelles of casein (plasmin and lipoprotein lipase). Other enzymes present are lactate dehydrogenase, malate dehydrogenase, lactoperoxidase, galactosyl transferase, alkaline phosphatase, phosphoprotein phosphatase, ribonuclease, lysozyme, fructose biphosphate aldolase, and glucose phosphate isomerase. Many enzymes in milk are original enzymes coming from the cow's udder.

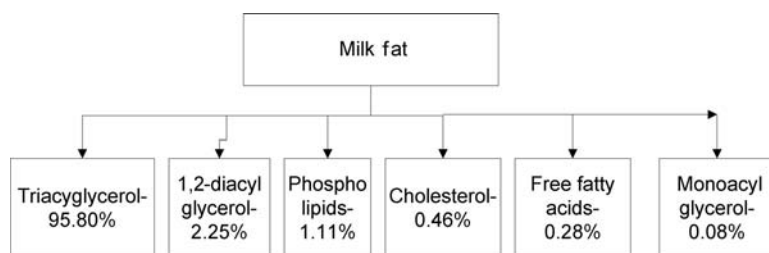


Figure 3.5. Major components of milk fat. Adapted from McGuire and Bauman (2003) and Chandan (2007a).

BIOSYNTHESIS OF MILK LIPIDS

Milk fat, even though quite bland in taste, imparts richness/smoothness to (fat containing) dairy products. Milk fat in freshly secreted milk occurs as microscopic globular emulsion of liquid fat in aqueous phase of milk plasma. Fat content of milk varies from 3.4 to 5.1%, depending on the breed of the cow. Most of the milk used in dairy processing typically contains an average of 3.5–3.6% fat. Variability of milk fat depends upon the individuality of animal, stage of lactation, feed, environmental factors, and stage of milking. Typical composition of milk fat in terms of major constituents is given in Figure 3.5.

The functional properties of milk fat are attributed to its fatty acid make up. 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. It contains 7–8% short chain fatty acids (C_4 – C_8), which is a unique characteristic of milk fat. The major fatty acid profile of milk fat is given in Table 3.5.

Milk fat functions as a concentrated source of energy as well as a source of fat-soluble vitamins A, D, E, and K and essential fatty acids, linoleic, and arachidonic acids. The essential fatty acids are not synthesized by human body. They must be supplied by the diet. Arachidonic acid with four double bonds is present in traces. Its precursor is linoleic acid. Omega-3-linoleic acid and its products EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are also present in trace but in significant amounts. The positional location of individual fatty acids in the triglycerides is not random, which illustrates the specificity of milk fat synthesis. The *syn*-1 and *syn*-2 positions on the glycerol molecule are mainly occupied by myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), or oleic acids ($C_{18:1}$). The *syn*-3 positions con-

Table 3.5. Fatty Acid Profile of Milk Fat

Fatty Acids	Common Name	Weight (%)
$C_{4:0}$	Butyric	3.8
$C_{6:0}$	Caproic	2.4
$C_{8:0}$	Caprylic	1.4
$C_{10:0}$	Capric	3.5
$C_{12:0}$	Lauric	4.6
$C_{14:0}$	Myristic	12.8
$C_{14:1}$	Myristoleic	1.6
$C_{15:0}$	—	1.1
$C_{16:0}$ (branched)	—	0.30
$C_{16:0}$	Palmitic	43.7
$C_{16:1}$	Palmitoleic	2.6
$C_{17:0}$	—	0.34
$C_{18:0}$ (branched)	—	0.35
$C_{18:0}$	Stearic	11.3
$C_{18:1}$	Oleic	27.42
$C_{18:2}$	Linoleic	1.5
$C_{18:3}$	Linolenic	0.59

Adapted from Chandan (2007a).

tain butanoic ($C_{4:0}$), hexanoic ($C_{6:0}$), or oleic ($C_{18:1}$) acids.

The biosynthesis of individual fatty acids and their comparative concentration in milk fat has a profound effect on properties and utilization of milk fat. Saturated fatty acids are solid at ambient temperature, while unsaturated fatty acids are liquid. Their ratio in milk fat has a significant effect on the hardness and spreadability of butter at refrigerated storage temperature. Furthermore, the balance between C_4 and C_{18} fatty acids keeps milk fat liquid at body temperature. There is a correlation between the fatty acid composition of feed lipids and butter hardness. A seasonal effect is seen as well. A softer butter is observed when the cow is on summer pasture or when the ration includes oils that are liquid at ambient temperature.

Cholesterol is a component of blood from where it enters milk pool. The cholesterol content of milk is significantly affected by the species, breed, feed, stage of lactation, and season of the year. Cholesterol content is generally lowest in the beginning of lactation period and progressively rises throughout the lactation period being highest toward the end of the lactation. The cholesterol content of colostrum is relatively high (570–1950 mg/100 g fat) for the first milking after parturition and progressively declines to normal levels (0.46 mg/100 g fat) during subsequent milking.

The fatty acids needed in the synthesis of triacylglycerol (triglycerides) come from two sources described below.

Blood plasma lipids originating from digestion and absorption of dietary fat as well as by mobilization from adipose tissue. More than 80% of blood serum lipids are derived from dietary sources. Milk yield and stage of lactation influence their concentration in blood. Approximately, 50% of fat fatty acids of milk owe their origin to blood lipids. In this regard, most of the C_{18} fatty acids and about 33% of C_{16} fatty acids originate from dietary fat. Dietary fats are composed of more unsaturated fatty acids than milk fat and adipose fat. The dietary fats consist mainly of long chain fatty acids, namely, palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$). The dietary fatty acids get biohydrogenated in the rumen by the bacteria to generate saturated fatty acids from unsaturated fatty acids. In ruminants, some of the saturated fatty acids, especially stearic converts to unsaturated oleic acid by an enzyme desaturase located in the epithelium of small intestine. However, bulk of desaturation takes place in the mammary epithelium. In this way, oleic acid content is enhanced, which confers fluidity to milk fat and thereby facilitating efficient secretion of milk from mammary gland.

De novo synthesis in the mammary epithelial cells utilizes acetate (C_2) and β -hydroxybutyrate (C_4) as sources of carbon. Nearly, all C_4 to C_{14} fatty acids are synthesized from these two precursors.

The acylglycerols or glycerides of milk are synthesized in the cytoplasm surface of the smooth endoplasmic reticulum of mammary epithelial cells, employing a key enzyme Acetyl CoA carboxylase. This enzyme becomes very active during lactogenesis. Milk lipids are synthesized via α -glycerol phosphate pathway. Two acyl CoA molecules react with α -glycerol-3-phosphate to form phosphatidic acid, which converts to 1,2-diacylglycerol upon removal

of the phosphate. An additional long chain acyl CoA adds the final fatty acid to form the triacylglycerol and CoA. Key steps involved in the biosynthesis of fat are summarized in Figure 3.6.

BIOSYNTHESIS OF MILK SUGAR, LACTOSE

Glucose is the exclusive monosaccharide substrate for lactose biosynthesis. In ruminants, 45–60% of blood glucose is formed from propionate in the liver by Gluconeogenesis process. It is interesting that blood glucose level in nonruminants is approximately double than in the blood of ruminants. Biosynthesis of lactose occurs in the membranes of Golgi apparatus. Two molecules of glucose give rise to one molecule of lactose. Glucose is converted to UDP-galactose by a cascade of several enzymatic reactions. At the onset of parturition, the enzyme activity shows a dramatic increase to cope up with lactogenesis (the lactation process). Glucose and UDP-galactose are combined to form lactose, catalyzed by the action of lactose synthase that is composed of galactosyl transferase and α -lactalbumin. The rate of lactose biosynthesis is determined by the availability of α -lactalbumin from the RER. Lactose cannot diffuse out of Golgi membrane as well as out of the secretory vesicles membrane. The nonpermeable property of lactose is important in that it draws water osmotically into the Golgi.

The major carbohydrate of milk, lactose monohydrate, ranges from 4.8 to 5.2%. Lactose content of milk is relatively constant. In colostrum and mastitic milk, its concentration is significantly lower. It is a disaccharide of one residue each of D-glucose and D-galactose. Structurally, lactose is 4-O- β -D-galactopyranosyl-D-glucopyranose. Fresh milk contains small amounts of glucose (100 mg/100 mL), galactose (100 mg/100 mL), and oligosaccharides (10 mg/100 mL).

Lactose is the most constant constituent of milk, providing and maintaining osmolality of milk during the formation and subsequent milk secretion. The biosynthesis of lactose is summarized in Figure 3.7.

SECRETION OF MILK CONSTITUENTS INTO LUMEN

In the preceding sections, we have seen how the major constituents of milk are formed. Milk constituents are individually synthesized inside the secretory cell. After they are transported to lumen space, they blend

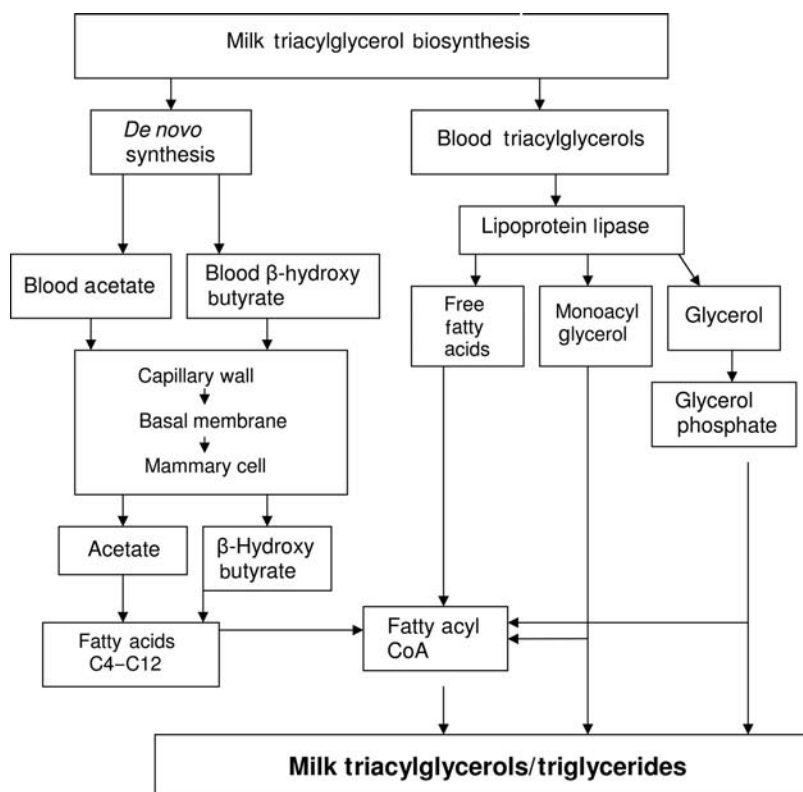


Figure 3.6. Steps in the biosynthesis of milk fat triglycerides.

together to form so-called milk. The process for secreting nonfat constituents differs from that of milk lipids. Milk proteins synthesized in the RER are incorporated into Golgi vacuoles or vesicles along with lactose and minerals. The secretory vesicles then separate from the Golgi apparatus and transport molecules toward the apical region of the cell. The membrane surrounding the vesicles fuses with the plasma membrane of epithelial cells followed by delivery into lumen space. At this point, major minerals are partitioned into colloidal and solution phase as shown in Table 3.6. Calcium and phosphate are associated with casein micelles. The physical state and particle size distribution in milk has been shown earlier in Table 3.3.

Milk lipids follow a discrete secretory process. As the molecules of synthesized milk fat transfer from the endoplasmic reticulum toward the apical membrane, their droplets grow in size. While passing through the apical membrane, they are pinched off as spherical globules with a coating of apical plasma

membrane. The FGM forms an envelope around fat particles. Figures 3.8 and Figure 3.9 illustrate the structure of milk fat globule, including the milk FGM.

MILK FGM

The fat globules are stabilized by a very thin membrane, closely resembling plasma membrane. The FGM is only 5–10 nm thick. The FGM consists of proteins, lipids, lipoproteins, phospholipids, cerebrosides, nucleic acids, enzymes, trace minerals and bound water, details of which are given in Table 3.7.

Most of the protein fraction associated with FGM originates from the membrane. The lipids originate from the walls of secretory vesicles carrying the non-fat components of the milk to the apical membrane. Certain enzymes, such as the alkaline phosphatase and xanthine oxidase as well as certain important minerals such as iron and copper are preferentially attached to the FGM. The membrane contains 5–25% of the total copper and 30–60% of total iron content

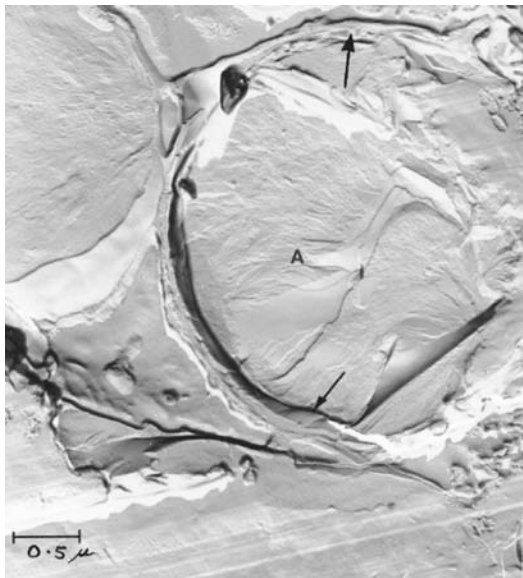


Figure 3.9. Electron micrograph of freeze-etched fat globule of unwashed cream obtained from Jersey cow milk ($\times 31,000$). Note the membrane as depicted by arrows and region A is milk fat core of the globule. Adapted from Chandan (2007a).

phospholipids and neutral lipids in the ratio of 2:1. The phospholipids are phosphatidylcholine (34% of total phosphorus), phosphatidylethanolamine (28%), sphingomyelin (22%), phosphatidyl inositol (10%), and phosphatidyl serine (6%) (Fox and McSweeney, 1998). The major fatty acid content of phospholipids is 5% C_{14:0}, 25% C_{16:0}, 14% C_{18:0}, 25% C_{18:1}, 9% C_{18:2}, 3% C_{22:0}, and 3% C_{24:0}. Accordingly, unsaturated content of the membrane lipids is different from rest of milk lipids in terms of their high unsaturated fatty acid level.

Table 3.7. Proximate Composition of Bovine Milk Fat Globule Membrane

Component	% (w/w) of Total Membrane
Protein	41
Phospholipids	27
Neutral glycerides	14
Water	13
Cerebrosides	3
Cholesterol	2

Adapted from Chandan (2007a).

The neutral lipids of the membrane consist of approximately 83–88% triglycerides, 5–14% diglycerides, and 1–5% free fatty acids. The fatty acids contained therein are largely long chain. In order of their preponderance, they are palmitic, stearic, myristic, oleic, and lauric acids.

The sterols, vitamin A, carotenoids, and squalene are largely located in the fat core of the globule.

RATE OF MILK SECRETION

Milk secretion rate is related to time since the previous milking of the cow. A low intraalveolar pressure is seen during the period following milking. Consequently, it facilitates the transport of newly synthesized milk into alveolar lumen. With secretion of more milk, a backpressure is created onto secretory cell by alveolar luminal contents. This process continues until the alveolar enlargement reaches its limit and the luminal pressure exceeds the force of secretion. When the pressure differential between the lumen and secretory forces becomes positive in favor of the lumen pressure, newly formed milk cannot be pushed out of secretory cell. Simultaneously, the build-up of newly secreted milk in the cells is accompanied by reduction of uptake of precursors by negative feedback or mechanical factors.

Following milking, as the time increases, the udder pressure continues to increase from 0 to 60 mm Hg after 30–35 hours. Simultaneously, the rate of secretion in the udder registers a decline from 1.2 kg/h to approximately 0.2 kg/h. After 10 hours from previous milking, the average secreting rate slows down and after 35 hours it stops completely. The udder pressure increase per unit of milk formed is lower for cows producing more milk as compared to cows producing lower milk output. Also, the pressure is lower for older cows than for younger cows. The stage of lactation influence the pressure, which is lower in early lactation than in late lactation.

For detailed discussion of milk biosynthesis and secretion, the reader is referred to excellent publications on the subject (Bauman et al., 2006; Keenan, 2003; McGuire and Bauman, 2003; Stelwagen, 2003a,b).

CURRENT AND FUTURE MAMMARY GLAND-BASED RESEARCH

Biosynthesis and high throughput ability of mammary epithelial cells has attracted considerable

research attention. Efforts are ongoing to exploit mammary gland cell biology to derive tangible benefit through sound science. Current mammary gland/epithelial cell-based research and its attendant food/pharmaceutical outcomes are summarized as below. The list is not meant to be all-inclusive but rather arbitrary. The main idea is to stimulate applied interest in next generation of scientists for food-pharmaceutical interface of dairy industry.

COMPOSITIONAL QUALITY

Since compositional quality could ultimately influence product characteristics, quality, yield, and nutritional status, there is a strong interest to elucidate fundamental relationship of milk production factors having influence on compositional quality (Carroll et al., 2006). The overall objective in such effort is to ultimately enhance profitability of dairy processing operations. However, variations in quality parameters and complex interdependence of production variables make it very challenging to establish predictable relationship. To address this, well-designed and controlled experiments are the best solution. For example, in New Zealand and Australia, pastoral farming is uniform (less feeding and management variations) and herd testing is carried out at central facility (less interlaboratory variations). Controlled experiments conducted on various milk cattle in the above set up gave meaningful comparisons (Boland, 2003).

There is a considerable variation in milk composition across various breeds despite years of selective breeding. The relative amounts of fat, protein, lactose, and water could be influenced by breed, genetics, diet, and other unknown environmental factors. From the standpoint of market value and nutritional importance, protein and fat content of milk are relatively more significant. These constituents can be manipulated by nutritional regime of the cow or by utilizing natural genetic variation (Givens and Shingfield 2003). With the availability of genomic and advanced technological tools, research in this area looks promising.

HEALTHY MILK FAT

Dietary fat is well known to influence fatty acid profile and ultimate composition of milk fat of dairy animals. Food fats that are rich in saturated fatty acids are implicated in increased risk of cardiovascular disease. In contrast, dietary intake of fats rich in

cis-monounsaturated fatty acids and long chain *n*-3 polyunsaturated fatty acids is shown to play helpful role in the prevention of heart disease. There is much interest in manipulating the fatty acid composition of milk fat by feeding cow with feed containing unsaturated fats that are shielded from saturation effects normally happening during the cow's digestive processes. The objective is to reduce the proportion of saturated fatty acid (lauric, myristic, and palmitic acid) content of milk fat and enhance the level of cis-monounsaturates and *n*-3 polyunsaturates. These efforts have met with some degree of success. Conjugated linoleic acids (CLA) are recognized as health-promoting fatty acids. Efforts have been made to modify milk fat composition toward CLA-rich milk. The level of CLA is dependent on substrate (vaccenic acid, also called trans-11 linoleic acid) and desaturase in the mammary epithelial cells. Various dietary supplements such as fish oil and oleamide have shown encouraging results. Looking to apparent interest in health-promoting fat composition of dairy foods, prospects are bright for more research. Enhancing fatty acid composition of milk fat to a commercially significant level would be beneficial to both consumers as well as milk producers who would get fair returns.

GENETIC POLYMORPHISM AND ENHANCING CHEESE QUALITY AND YIELD

Genetic polymorphism is a result of change in the protein structure and associated function due to point mutation in the corresponding DNA transcript. Relative concentration of κ -casein is important in cheese yield and quality. Higher level of κ -casein B is significant in Jersey cattle compared to other breeds. It has been found that due to polymorphism in κ -casein site, BB phenotype cows showed higher concentration of κ -casein that ultimately resulted into higher total casein and cheese yield. More definitive studies are required to elucidate if known milk protein polymorphisms have genetic basis for plausible influence on product quality. Then, it may be practical to design breeding strategy destined for special milk production.

In a small pilot scale study in Kaikoura dairy cooperative in New Zealand, it was found that cows having BB polymorphism in β -lactoglobulin tended to have higher percentage of casein compared to AA milk. It would be interesting to see whether selective breeding for B variant of β -lactoglobulin could provide consistently higher cheese yield.

BIOACTIVE PEPTIDES

Since time immemorial, milk and milk products have provided nutritional and energy needs for the milk consuming consumer. However, there is now increasing realization that diet could influence health. As a result, health-promoting benefits are sought by modern consumer in their food menu. In this context, bioactive properties displayed by some proteins, peptides, and fatty acids in milk has attracted strong research interest (Aimutis, 2004). Among milk components, CLA, vaccenic acid, and sphingolipids are thought to be associated with anticancer properties. Also, CLA, stearic acid, and omega-3 fatty acids are associated with improved cardiovascular health. Antihypertensive peptides (α -s₁-casokinin, β -casokinin, and β -lactorphin) in milk are associated with cardiovascular health through inhibition of angiotensin converting enzyme (Akers, 2002). Similarly, immunostimulatory, antithrombotic, antimicrobial, and opioid antagonistic activities of milk protein fractions are beginning to be understood. CLA along with calcium, phosphorus, and probiotics tends to improve bone health and immune protection. It is reasonable to conjecture that new era of functional foods and health awareness would continue to provide impetus to drive research in this fascinating biopharma interface of dairy industry.

FIGHTING INTRAMAMMARY INFECTIONS

Since udder health is important for uniform compositional quality of milk, efforts are ongoing to enhance mastitis control and prevention strategies. Good on-farm mastitis control measures have addressed contagious mastitis problem; however, environmental mastitis attributed to *Streptococcus uberis* and *Escherichia coli* remain a challenge. Immunotherapeutic strategy employing efficacious and specific protective antibody in mammary mucosa holds considerable promise (Mastitis laboratory, University of Tennessee). Researchers at the University of Vermont have enabled mammary gland cells to secrete antibacterial factor lysostaphin, which is antibacterial for *Staphylococcus aureus*. Initial studies with *Staphylococcus aureus* challenge are encouraging (Kerr and Wellnitz, 2003). However, application of transgenic animals and secreted transgene product is being further evaluated for safety.

In general, human society has not yet resolved moral/ethical dilemma of biotechnology-derived products/applications. If safety of cloned meat

(Sundlof, 2006) or skin-derived stem cell (nonembryo) research (Yu et al., 2007) is any indication, it may be anticipated that environment for research in biotechnology arena would continue to receive due attention. It might be predicted that novel application in mammary gland-based research would continue to gain attention to fully exploit nature's bioprocessing factory.

CONCLUSIONS

Mammary gland is a dynamic site where milk biosynthesis occurs. Individual milk constituents are synthesized in mammary epithelial cells by distinct pathways most of which are understood now. After individual synthesis and after undergoing structural changes, milk components are transported to alveolar lumen. Milk proteins are distinct class of dietary proteins in that they have biologically superior amino acid profile and nutritional value, and possess bioactive properties in comparison with other dietary sources of proteins. Milk fat and its fatty acid profile are influenced by diet. Research work has shown that the fatty acid profile of milk fat could be altered to reduce its saturated fatty acid content in order to develop a heart healthy milk fat without atherosclerosis effects.

CLA found in milk fat are associated with beneficial health attributes. For example, CLA has positive influence on cardiovascular health. They possess anticancer activity and might be useful in weight management and obesity control. Accordingly, research interest is continuing to manipulate cow diet to boost CLA production in milk. Manipulation of cow diet to take advantage of rumen fermentation and mammary gland desaturase regulation to ultimately enhance CLA profile of milk fat looks promising.

Dairy scientists are also looking to exploit compositional quality profile of milk including milk protein polymorphism to enhance technological properties of milk and milk products with a view to increase cheese quality and yield. Consumer interest in functional dairy foods should encourage further research in underexplored dairy foods/biopharma area with potential benefit for both consumers as well as milk producers. Milk is traditionally perceived as a natural food. As a result, bioprocessed milk/milk products are not emotionally acceptable to the consumer. It is our optimistic forecast that positive health benefit of bioprocessed milk and dairy foods based on science and clinical studies focusing on demonstratable food safety should foster conducive environment for more

exciting research in mammary gland biology and safe bioprocessing.

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4

Chemical Composition, Physical and Functional Properties of Milk and Milk Ingredients

Kasipathy Kailasapathy

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Definition

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Lactose

Caseins

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Milk protein hydrolysates

α -Lactalbumin and β -lactoglobulin

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Immunoglobulin

Bioactive peptides

Specific Lipids

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Lactosucrose

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Physical Structure

Physical properties

Oxidation–Reduction Potential

Surface Properties

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Quality and safety tests and future trends

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Bibliography

DEFINITION OF MILK AND SAFE PROCESSING OF MILK AT THE FARM

MILK AS A FOOD

Milk is one of the few foodstuffs consumed without further processing, and it is generally regarded as the most nearly perfect food. Nutritionists worldwide agree that it is of enormous value in promoting growth and development of children and young animals. Although it is important for infant and childhood feeding, milk (and milk products) continues to be important in our diets right throughout our adult life.

A number of uses have been found for milk in the diet, ranging from infant feeding and nourishment, an additive to beverages such as tea and coffee, an ingredient for processed foods (e.g., sweets, confectionery, bread, pastry goods) and as the primary ingredient in manufactured dairy products (e.g., ice cream, cheese, yogurt, and butter). Milk may be processed and stored in some form (e.g., milk powder, condensed milk) and in order to overcome perishable nature of milk in its natural state.

DEFINITION

The term “market milk” refers to fluid whole milk that is sold to consumers usually for direct consumption. It excludes milk consumed on the farm and that used for the manufacture of dairy products. Milk may be defined as the whole, fresh, clean, lacteal secretion obtained by complete milking of one or more healthy milch animals, excluding that obtained within 15 days before parturition and 15 days after calving

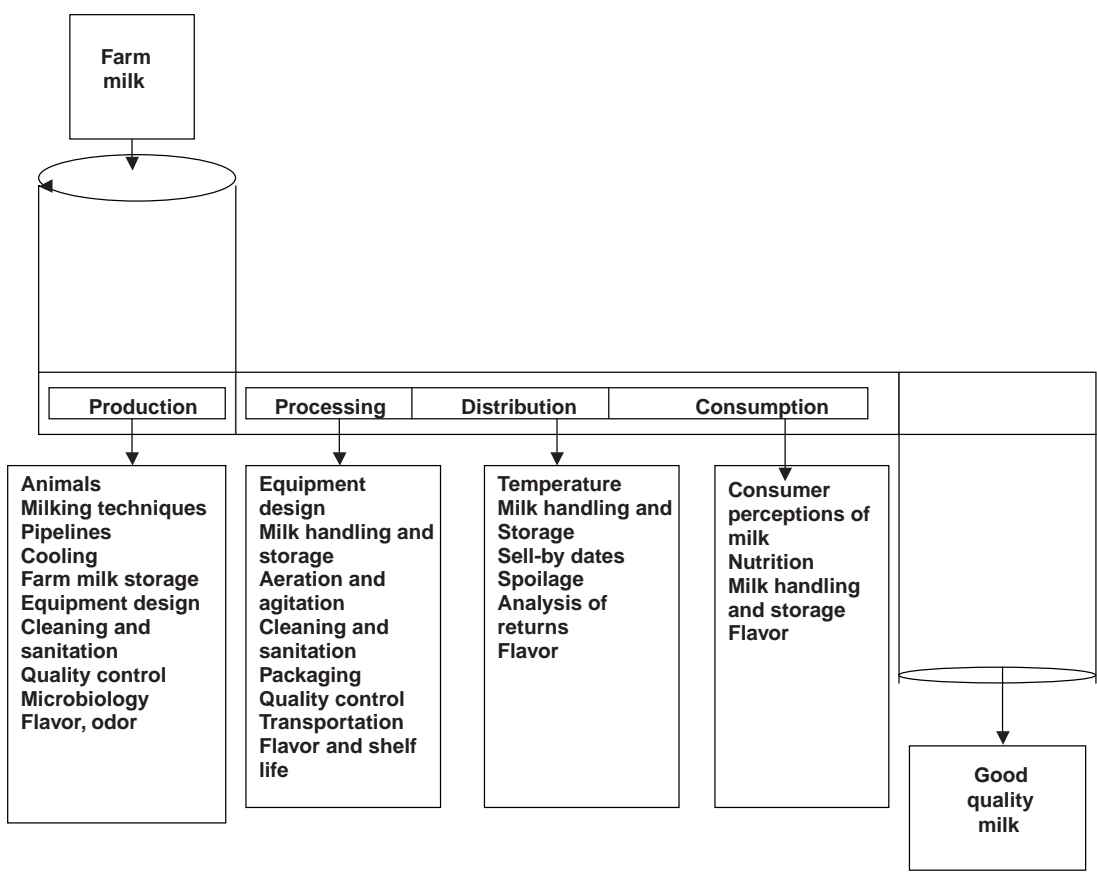


Figure 4.1. Factors that may affect milk quality.

or such periods as may be necessary to render the milk practically colostrum-free, and containing the minimum-prescribed percentages of milk fat and milk solids-not-fat (SNF).

MILK AND PUBLIC HEALTH

It is well known that milk can be a potential carrier of disease-producing organisms. Milk-borne epidemics have been reported widely. Proper precautions are necessary to prevent outbreaks of milk-borne diseases, especially if raw milk is consumed. Figure 4.1 illustrates the factors affecting milk quality at various stages.

Diseases can be transmitted through milk, some examples are listed below:

1. *Infection of milk directly from the cow:* This includes bovine disease, the causative organisms

may enter milk through mammary glands and/or through fecal contamination, and may cause a disease condition in persons who consume the milk. Examples include bovine tuberculosis and undulant fever.

2. *Infection from man to cow and then to milk:* These diseases are essentially human's, but can become established in the cow's udders. Examples include septic sore throat, scarlet fever, and diphtheria.

3. *Direct contamination of milk by human beings:* These diseases may be transmitted to the milk by direct contamination through human contact. Examples include typhoid fever, dysentery, gastroenteritis, and diphtheria.

4. *Indirect contamination of milk by human beings:* These include human illnesses; the pathogen causing the human illness may enter milk through contaminating utensils in the milking barn at the farm, water supply, or through insects (carriers)

Table 4.1. Sources of Contamination of Milk and Their Control

Sources of Contamination	Control Measures
Interior of udder	Check for mastitis, discard fore milk
Exterior of the cow, particularly udder and flank	Wash and wipe udder, clip udder and flanks dry milking
Barn air and dust	Keep milk covered
Flies and other vermin	Eliminate breeding of flies fl control with fl trap
The milker (hand milking)	Clean habits, dry milking
Machine milking	Sanitation of the milking machine, CIP
Utensils	Clean, sanitize, and dry before use

and dust. Examples include typhoid or paratyphoid fever and diarrhea.

MILK HARVESTING

Before milking commences, it is therefore important that infected cows be excluded. Cows that have mastitis, for example, will yield milk that is already infected with microorganisms and this milk will contaminate milk from healthy cows.

The sources of contamination of raw milk and their control are shown in Table 4.1.

Bacteria adversely affect milk quality. The methods and thoroughness of cleaning and sanitizing milking equipment are important factors that affect bacterial counts in milk. The rubber parts of the milking machine are especially troublesome to clean. Milk solids, especially fat accumulate in the pores of the rubber and provide an excellent nutrient medium for bacteria. Effective procedures to eliminate this problem include rinsing with lukewarm water, brushing with hot alkaline detergent solution, rinsing and then treating all utensils that come in contact with milk with a sanitizing solution. Rinsing equipment immediately after milking is especially important in effective sanitization of milking equipment. The vacuum line should also be cleaned periodically. It should be given an alkaline as well as an acid wash.

A properly operating milking machine should milk cows efficiently without irritating the udder. Signs of malfunctioning milking system include teat cups falling off, excessive vacuum fluctuations flood milk lines with uneven milk flow, slow return of vacuum level after an air leak, and slow milking.

Milk leaves the udder at a temperature of 37°C. Fresh milk from a healthy cow is practically free from

bacteria, but it must be protected against contamination as soon as it leaves the udder. Microorganisms capable of spoiling the milk are ubiquitous; they are found on the udder, the milker's hand, air-borne dust particles and water droplets, straw and chaff, and on cow hair and in the soil.

RAW MILK QUALITY

a. *Physical contamination:* By far the most important physical aspect associated with milk is the temperature from the time of milking to the time of receipt at the chilling or processing plant. Small suppliers use ice added to the milk to reduce temperature quickly. The practice of cooling milk by addition of ice dilutes milk, which can make processing difficult. The water from melting ice lowers the concentration of the dissolved and suspended components of milk and alters its processing properties. Poor quality water used to make ice or for rinsing milk utensils in the farm and factory can increase the bacterial counts in the milk. Dust from milking parlors and transport containers can spoil the milk. Poor-fitting lids on milk containers offer little protection from dust contamination during transport. Animal hair is also common in milk obtained by hand milking. Udder hair should be kept clean, and should be clipped short where it is close to teats.

b. *Chemical contamination:* The main chemicals that can contaminate milk are antibiotics, sanitizers, and detergent or soaps. After treatment of animals to control mastitis, milk is often withheld for too little time. Milk should not be used for consumption for at least 96 hours. Intramammary infusions of penicillin and other antibiotics are now commonly used. The time of withholding milk

from treated animals depend on the amount used and frequency of treatment, and the type of antibiotics. Antibiotics are undesirable in milk from the public health view point. Also, the presence of antibiotics in milk precludes its use from cultured products such as yogurts.

Sanitizing agents such as iodophors, quaternary ammonium compounds, and hypochlorites should be excluded from the milk supply. These are not normally a problem with small holders due to small amounts used. However, with large holders, who use machine milking, a breakdown can result in considerable quantities of sanitizers being included in the bulk milk. Poor rinsing practices after cleaning can result in detergent and soap residues in milk.

c. *Biological contamination*: Large numbers of microorganisms in milk are undesirable for public health and spoilage reasons. Pathogenic organisms can be secreted in the milk from cows suffering from a variety of diseases such as tuberculosis and brucellosis. Spoilage organisms multiply rapidly if the temperature of milk remains above 10°C. While they are not a great public health threat, acid produced by spoilage organisms can render milk unfit for heat treatment or processing. Acid (sour) milk curdles on heating and can easily block plate heat exchangers and other equipment in the processing factory. Other microorganisms such as yeasts and moulds are able to cause quality problems by producing off-flavours. Ice made from untreated river water may contain high levels of microorganisms. Microorganisms are not killed by freezing and if ice is used for cooling milk, the milk gets contaminated immediately. Most of the microorganism in milk originates from contaminated milking utensils. Use of detergent for cleaning, scrubbing, rinsing with clean hot water and drying in a clean environment after use are essential. Milker's hand should be washed before milking. Udder and teats of cows should be clean and dry. Where animals have wet or dirty udders, the teats and surrounding areas should be cleaned, sprayed, or rubbed with a sanitizing solution, and dried before milking.

RAW MILK QUALITY AT THE RECEIVING BAY IN A PROCESSING PLANT

Milk should be inspected immediately on receipt. There are four categories of inspection: visual, organoleptic, chemical, and microbiological.

Visual: Milk should be free from extraneous matter such as dust, insects, and should be normal white or white-yellow color dependent on the breed of animal. Sediment and suspended particles can be detected by means of a sediment tester.

Organoleptic: This includes smell, appearance, and taste. A check of the container for milk particles after emptying is important.

Chemical: Most important chemical test on receipt is for developed acidity. Milk with developed acidity will clot on heating and block processing equipment.

Microbiological: Plate counts for standard plate counts (SPC) and direct microscopic count are used to assess the microbiological quality of milk.

MILK COMPOSITION

Milk is the whole, fresh, clean lacteal secretion obtained by the complete milking of one or more healthy cows. Milk shall contain not less than 3% milk fat, and not less than 8.25% milk SNF. Milk may be standardized by the addition or removal of cream or by the addition of skim milk. When so standardized, milk shall contain not less than 3.25% milk fat, and not less than 8.25% of milk SNF. Milk must not be used for processing 15 days before and 5 days after calving.

The composition of milk differs between different mammals, and between different breeds of the same species. Table 4.2 shows an illustration of the typical composition of milk of different mammals.

Table 4.3 illustrates the differences in composition between cow's and buffalo's milks.

CONSTITUENTS OF MILK

The constituents of milk may be listed diagrammatically as shown in Figure 4.2

Table 4.2. Typical Chemical Composition of Milk of Different Species (% Composition)

Species	Water	Fat	Protein	Lactose	Ash
Ass	90.0	1.3	1.7	6.5	0.5
Buffalo	84.2	6.6	3.2	5.2	0.8
Camel	86.5	3.1	4.0	5.6	0.6
Cow	86.6	4.6	3.4	4.9	0.5
Ewe	79.4	8.6	6.7	4.3	1.0
Goat	86.5	4.5	3.5	4.7	0.8
Human	87.7	3.6	1.8	6.8	0.1
Mare	89.1	1.6	2.7	6.1	0.5

Table 4.3. Physicochemical Characteristics of Cow and Buffalo Milks

Characteristics	Buffalo Milk	Cow Milk
PH	6.7000	6.6000
Buffer value (pH 5.1)	0.0417	0.0359
Density at 20°C	1.0310	1.0287
Viscosity (cP)	2.0400	1.8600
Specific refractive index	0.2061	0.2059
Surface tension	5.4000	5.5900
Acidity (%)	0.1500	0.1400
Fat globule size (μm)	5.0100	3.8500
Phosphatase (units)	28.000	83.000
UV fluorescence	Greenish-yellow	Pale-bluish

Water

Water is the medium in which all the other components of milk (total solids) are dissolved or suspended. Small amounts of water in milk are hydrated or bound chemically to lactose and salts, and some are bound to proteins. Removal of water from milk such as in concentrated and dried milk products increases its shelf life by reducing water activity. During cheese making, some of the original water content of milk is removed in the form of whey. The water remaining in the curd and cheese furnishes suitable conditions for the chemical and biological reactions upon which cheese making is based, provides moisture which is essential for the growth and activities of microorganisms and the amount of remaining water in the product influences flavor, body, texture, color, and appearance of the finished product. Regulation prohibits addition of water to raw milk and, also, there is maximum moisture content established for cheese.

Milk Lipids

Milk fat is the most variable of all the milk constituents. It is mainly a mixture of triglycerides (TG). The other 1–2% of milk fat is composed of phospholipids, steroids, carotenoids, and fat soluble vitamins A, D, E, and K. The average composition for milk lipids is shown in Table 4.4.

Milk fat exists in milk in small globules as an emulsion. These globules are 1–20 μm in size. Each fat globule is surrounded by an adsorbed layer of other milk constituents, mainly proteins and phospholipids. This stabilizes the fat emulsion and prevents fat from separating out. Because the fat is of

lower density than the remainder of the milk, the fat globules tend to rise to the surface, when the milk is allowed to stand undisturbed, giving a cream layer on top of the milk. This property is made use of in the production of cream. Mechanical separators using centrifugal force separate the fat much more efficiently. Homogenized milk is produced by pumping the milk through a tiny orifice at very high pressures. This breaks down the fat globules to much smaller size which prevents their separation.

Milk fat is important in cheese making because it is directly related to the yield of cheese, used to establish the price of milk paid to the farmer, contributes to flavor of cheese and to the body characteristics of cheeses. Milk lipids become rancid when lipases hydrolyze them releasing short-chain fatty acids. Factors that contribute to rancidity in milk and milk products include improper homogenization of raw milk or mixing homogenized milk with raw milk, excessive agitation of warm raw milk (foaming), thermal activation (re-warming of previously cooled raw milk), freezing of milk, and excessive growth of psychrotrophs.

The main constituent of milk lipids is triacylglycerol with much smaller quantities of sterols and phospholipids which are associated with the membrane (Table 4.4). The sterols are mostly cholesterol with about 10% of this is in the ester form. Traces of hydrocarbon, carotenoids, retinyl esters, and squalene are found in freshly drawn and extracted or processed milks. Traces of free fatty acids (FFA) and di- and monoacyl glycerols (DG and MG) are present in milk lipids. The presence of greater amounts of these and smaller amounts of TG is indicative of lipolysis. Lipolysis will alter the relative amounts of FFA, TG, DG, and MG.

The structure of the TG influences the action of lipolytic enzymes and, therefore, absorption and flavor of cheeses. Structure of milk is recognizable for the melting point, crystallization behavior, and rheological properties of milk fat as globules, and in butter and butter oil. Bovine milk lipids contain about 12 fatty acids in amounts greater than 1% (Table 4.5).

The TG are characterized by the location of most of the 4:0–8:0 fatty acids at the Sn-3 position and 12:0, 14:0, and 16:0 at the Sn-2 position. Structure affects the behavior of milk lipids during and after processing, the metabolism of milk lipids, and possibly their hyper-cholesterolemia potential.

Phospholipids and sphingolipids are minor components of milk lipids. During high-temperature processing such as UHT pasteurization, phospholipids are destroyed due to auto-oxidation of the

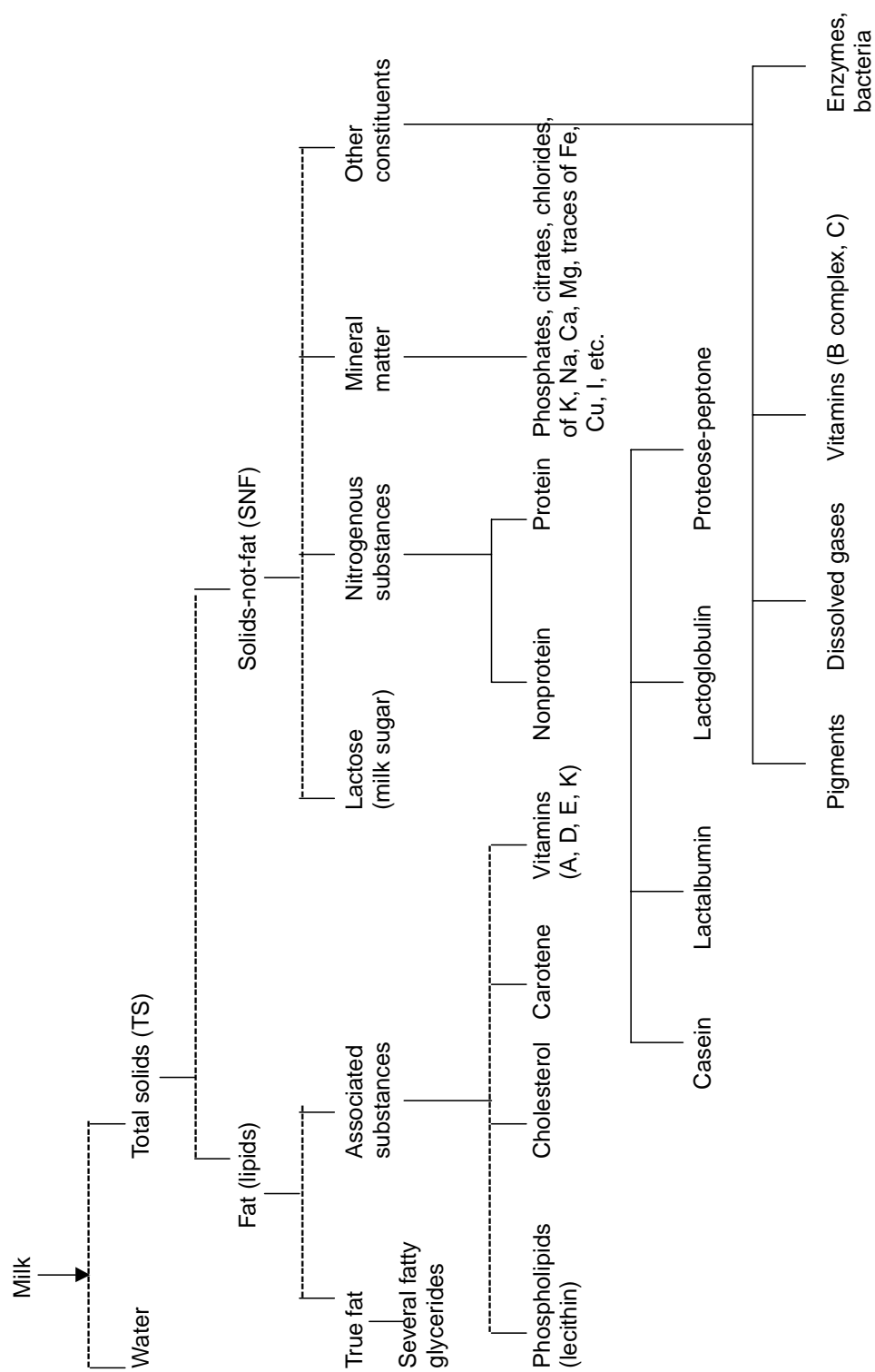


Figure 4.2. Milk constituents.

Table 4.4. Lipids Classes in Bovine Milk

Lipid Class	% Total Lipid (g/100 g)
Phospholipid	1.11
Cholesterol	0.46
Triacylglycerol	95.80
1,2-Diacylglycerol	2.25
Free fatty acids	0.28
Monoacylglycerol	0.08
Cholesteryl ester	0.02

Table 4.5. Positional Distribution of Fatty Acids in the Triacylglycerols in Bovine Milk

Fatty Acids	Triacylglycerol	Sn-1	Sn-2	Sn-3
4:0	11.8	—	—	35.4
6:0	4.6	—	0.9	12.9
8:0	1.9	1.4	0.7	3.6
10:0	3.7	1.9	3.0	6.2
12:0	3.9	4.9	6.2	0.6
14:0	11.2	9.7	17.5	6.4
15:0	2.1	2.0	2.9	1.4
16:0	23.9	34.0	32.3	5.4
16:1	2.6	2.8	3.6	1.4
17:0	0.8	1.3	1.0	0.1
18:0	7.0	10.3	9.5	1.2
18:1	24.0	30.0	18.9	23.1
18:2	2.5	1.7	3.5	2.3

polyunsaturated fatty acid (PUFA) in the phospholipids as a result of exposure to heat during processing. Hence, powdered whole milk and butter milk contain very little or no phospholipids. The phospholipids and sphingolipids bind cations, help stabilize emulsion, and probably orient enzymes on the globule surface, but their effects in processed milks are unknown.

Milk contains 10–20 mg/dL of cholesterol or 308–606 mg/100 g of fat in whole milk containing 3.3% fat. This amount is positively correlated with the fat content of dairy products (Table 4.6).

Cholesterol is the major sterol and is located mostly in the milk lipid globule membrane. About 10% of the cholesterol is esterified. Fatty acid composition of a reference-milk is shown in Table 4.7.

Factors that affect fatty acid composition include animal, genetic and stage of lactation, feed, grain amount, and composition of dietary fat, dietary protein, and seasonal and regional effects. The influence of all these factors except seasonal and regional ef-

Table 4.6. The Cholesterol Content of Various Dairy Products

Product	Fat (%)	Cholesterol (mg/100 g)
Skim milk	0.25	2
Whole milk	3.30	14
Cream	34.87	110
Cottage cheese (creamed)	4.51	15
Blue cheese	28.74	75
Ice cream	10.77	45
Swiss	27.45	92
Cheddar	33.14	105
Butter	81.11	219

Table 4.7. Fatty Acid Composition of Bovine Milk Fat

Fatty Acid	Common Name	Weight (%)
4:0	Butyric acid	3.32
6:0	Caproic acid	2.34
8:0	Caprylic acid	1.19
10:0	Capric acid	2.81
12:0	Lauric acid	3.39
14:0	Myristic acid	11.41
14:1	Myristoleic acid	2.63
16:0	Palmitic acid	29.53
16:1	Palmitoleic acid	3.38
18:0	Stearic acid	9.84
18:1	Oleic acid	27.39
18:2	Linoleic acid	2.78
18:3	Linolenic acid	0.59

fects is eliminated by the pooling of milk. The types of fatty acids in milk include saturated and branched chain fatty acids, monounsaturated fatty acids (oleic acid: *cis*-9–18:1), and PUFAs. Although dairy cattle consume relatively large amount of PUFA, the amount in milk is low because of ruminal biohydrogenation. Free volatile short-chain fatty acids, *n*- and branched-chain, contribute to the characteristic flavors of ripened cheeses. However, 4:0, and to a lesser extent, 6:0–10:0, can produce an extremely unpleasant flavor in raw milk when the lipoprotein lipase therein is activated. This can result when excessive foaming or agitation of raw milk occurs. The 2-oxo and 4- and 5-hydroxy acids are precursors of methyl ketones and γ - or δ -lactones which contribute to flavor as do the aldehydes resulting from the oxidation of unsaturated fatty acids.

Table 4.8. Protein Composition of Bovine Milk

Protein Components	Weight (g/kg)
Total protein	35.1
Total casein	28.6
Whey protein	6.1
α -s ₁ -Casein	11.5
α -s ₂ -Casein	3.0
β -Casein	9.5
κ -Casein	3.4
γ -Casein	1.2
α -Lactoglobulin	1.2
β -Lactoglobulin	3.1
Serum albumin	0.4
Immunoglobulin	0.8
Proteose-peptones	1.0

Milk Proteins

The proteins in milk are partly in solution and partly in colloidal suspension. The protein composition of mature herd milk is shown in Table 4.8.

Milk proteins are of two distinct types, whey protein (serum proteins) and caseins. Casein constitutes over 80% of the total protein in milk, although the relative proportion of whey protein to casein varies according to the stage of lactation. Caseins are subdivided into five main classes: α -s₁, α -s₂, β , γ , and κ -caseins. Caseins are globular protein with phosphoserine residue which provide them with unique properties. Phosphoserine residues are concentrated in clusters and are responsible for the existence of hydrophobic areas of strong negative charges. The molecules also contain blocks of hydrophobic residues. β -Casein contains the most hydrophobic component and forms aggregates with the N-terminal hydrophilic parts exposed to solvent and hydrophobic parts in the interior. α -s-Caseins are sensitive to calcium due to the presence of phosphate groups and precipitates in the presence of calcium ions at a pH of 7.0. κ -Casein differs from α - and β -caseins in having only one phosphoserine group and in containing a charged oligosaccharide moiety. The κ -casein molecule appears to consist of a relatively stable, single disulphide-bonded structure within which are both α -helical and β -sheet regions. The chymosin-sensitive Phe₁₀₅–Met₁₀₆ bond is thought to protrude from the molecular surface. One third of the κ -casein molecule is represented by the strongly ionic C-terminal section, which contains the three-oligosaccharide residues. The remainder of

the molecule is highly hydrophobic and corresponds to the para-k-casein formed after hydrolysis of the Phe–Met bond.

The amphiphilic nature of caseins and their phosphorylation facilitate interaction with each other and with calcium phosphate to form highly hydrated spherical complexes known as micelles. The casein micelle consists of an aggregate of almost spherical sub-micelles, which in turn consist of more limited aggregates of casein molecules. The calcium phosphate and α -s and β -casein are linked by the involvement of the phosphoserine residues in the structure of the calcium phosphate. κ -Casein is localized on, or very close to, the surface of the casein micelle. The hydrophobic part of the casein molecule is bound to the core of the micelle, while the hydrophobic macropeptide forms a layer of highly hydrated “hairs,” which projects into the aqueous phase. κ -Casein hairs are responsible for the steric stabilization of the casein micelles.

Whey protein comprises β -lactoglobulin and α -lactalbumins, proteose-peptones (partially derived from hydrolysis of β -caseins) and small quantities of the blood-derived proteins, serum albumin, and immunoglobulins. Whey proteins are typical compact globular proteins, with a relatively uniform sequence distribution of nonpolar, polar, and charged residues. These proteins undergo intramolecular folding as a result of the formation of disulphide bonds between cysteinyl residues, which buries most of the hydrophobic residues in the interior of the molecule. For this reason, whey proteins do not aggregate strongly, or interact with other proteins in the native state.

The major whey protein, β -lactoglobulin, undergoes limited self-association, at milk pH values, to form a dimer with a geometry resembling two impinging spheres. The dimer dissociates in a solution at 60°C, thus becoming susceptible to denaturation by unfolding of the tertiary structure. α -Lactalbumin, however, is more heat stable than β -lactoglobulin.

Effect of Heat on Milk Proteins. Casein micelles are remarkably stable at a temperature up to 140°C. In contrast, whey proteins are relatively heat-labile, extensive denaturation occurring at 80°C. β -Lactoglobulin is more heat-labile than α -lactalbumin as a consequence of its one free sulphohydryl group, which permits the initiation of autocatalytic disulphide exchange reactions.

Milk Proteins During Processing. The most important reactions of the milk proteins are those which

involve destabilization of the protein micelle. In some cases, these are technologically desirable reactions, such as the formation of a gel either when the pH of milk is reduced (e.g., manufacture of fermented milks and acid-set cheese), or when κ -casein undergoes selective proteolysis (e.g., manufacture of Cheddar cheese). Under the correct conditions, acidification may also be used to fractionate the milk proteins (e.g., manufacture of acid casein). In other cases, however, reactions involving destabilization of micelles are technologically undesirable. Examples include the various reactions involving aggregation of casein which occur during age thickening of UHT-treated milks and concentrated milks.

In the case of casein, the amphiphilic nature of the molecules, with the amino acids falling into hydrophilic and hydrophobic domains, imparts extremely good surface-active properties, and thus the functional properties of whipping/foaming and emulsification. Whey proteins are not amphiphilic in nature and are generally of lower surface activity than casein. Foam stabilizing properties are, however, superior since more rigid film is formed at the air/water interphase.

Lactose

Lactose is the major carbohydrate in milk and its concentration varies with milk yield between 4.2 and 5%, the lactose content usually being lowest in late lactation milk and in milk from animals having mastitis. Lactose is a disaccharide and comprises α -D-glucose and β -D molecules. Three solid forms of lactose exist, α -lactose monohydrate, anhydrous α -, and β -lactose. The β -form is of markedly higher solubility but, through mutarotation, an equilibrium mixture of the two forms exists in solution. In isolated form, lactose exists in either of the two crystalline forms, α -hydrate and anhydrous- β or as amorphous "glass" mixtures of α - and β -lactose.

Lactose is a reducing sugar and undergoes Maillard reactions with amino acids in milk resulting in brownish or burnt color of milk. Lactose is one of the least soluble of the common sugars, having solubility in water of only 17.8% at 25°C. This low solubility has consequences during the production of concentrated milk and frozen dairy products and it is sometimes necessary to induce crystallization to produce a large number of small crystals in order to avoid the "sandiness" defect. The α -hydrate crystal form, which is commonly formed, has large number of shapes, which causes the "sandiness."

Lactose has an asymmetric carbon in its structure and hence has optical properties. Lactose anomers rotate plane-polarized light and their concentration can be determined by polarimetric measurements. The α -lactose anomer is more dextra-rotatory than β -lactose. During crystallization, the β -form mutarotates to α -lactose. Crystals of α -hydrate form, which is most commonly formed are shaped like tomahawk and other shapes arise as a result of cocrystallization.

Lactose has poor solubility. As a general rule, a concentration of lactose exceeding 13 g/100 mL water in a dairy product tends to promote crystallization and a sandy texture results. During rapid drying, amorphous lactose is formed. This form of lactose is very hygroscopic and causes caking in dried products containing moisture levels of 8% or more. Under such conditions, the conversion of lactose glass to α -lactose-monohydrate crystals is responsible for binding powder particles as a "cake."

Lactose is only 25% as sweet as sucrose. Lactose finds its use as a food ingredient due to its protein stabilizing properties and low relative sweetness. Lactose may also be used as a partial replacer for sucrose in icings and toppings to improve mouthfeel without excessive sweetness. In baking industry, lactose imparts crust color (browning) and flavor due to caramelization. In formulated powdered product, the lactose crystals are slow to take up moisture, hence less caking or lumping. The pharmaceutical industry has used lactose as a processing aid in tableting for many years. Hydrolyzed lactose may contribute to sweetness. Lactose-hydrolyzed syrup from permeates and whey are used in confectionery and ice creams.

Lactose makes a major contribution to the colligative properties of milk: osmotic pressure, freezing point depression, and boiling point elevation. Lactose, for example, accounts for about 50% of the osmotic pressure of milk. Changes in the lactose content of milk are associated with reciprocal changes in the content of other water-soluble constituents, especially sodium and chloride.

When lactose is heated, it undergoes dehydration to form lactulose. It stimulates the growth of *Bifidobacterium bifidum* and is thus beneficial in establishing healthy commensal microbiota in the gut. Lactose is a good source of energy and may promote calcium absorption. Digestion of lactose presents a problem in some people as they lack β -D-galactosidase enzyme in their GIT. Consequently, dietary lactose is not hydrolyzed and reaches colon where it is metabolized by colonic bacteria forming gases (methanone and hydrogen). Accumulation of gas leads

to discomfort caused by bloating and diarrhea. Such lactose malabsorption is alleviated by yogurt containing live cultures, because the culture furnishes the lactose-hydrolyzing enzyme β -D-galactosidase and normal digestion pattern is restored. Lactase (enzyme that hydrolyze lactose) deficiency is most common in people from Africa or Asian origin, but can affect members of any other racial group. The degree of lactose intolerance varies among people and symptoms vary. Commercial processes for hydrolysis of lactose in milk and other dairy products have been developed in response to this problem.

Minerals

The minerals in milk consist principally of bicarbonates, chlorides, citrates, and bicarbonates of calcium, magnesium, potassium, and sodium. Most of the minerals are distributed between a soluble phase and a colloidal phase, as much as 60% calcium and 50% phosphorous may be in colloidal phase. The distribution of calcium, citrate, magnesium, and phosphate between soluble and colloidal phases and their interaction with milk proteins have important consequences for the stability of milk and milk products. Table 4.9 provides the approximate composition of the minerals in milk.

The minerals are present in a complex equilibrium consisting of colloidal state and soluble state. The ratio of colloidal to soluble state can influence the following characteristics:

- 1. Heat stability and alcohol coagulation of raw milk.
- 2. Quality and storage stability of concentrated, dried, or evaporated products.
- 3. Aggregate of fat globules during homogenization of milk fat.

Table 4.9. Partition of Major Minerals in Colloidal and Soluble Phases (% of Total Minerals)

Mineral	Colloidal	Dissolved
Calcium	67	33
Magnesium	36	64
Sodium	4	96
Potassium	6	94
Phosphate	55	45
Citrate	6	94
Chloride	0	100
Sulphate	0	100

- 4. Calcium content of milk influence firmness of curd during cheese making and viscosity of fermented milks.

The citrate concentration of milk varies according to season and the diet of the cow. The citrate concentration, in turn, can affect the soluble calcium content and milk stability. This has consequences for milk processing and may require the addition of anions to complex to ionic calcium, to reduce calcium available for binding to casein and stabilize milk against aggregation.

Minerals such as sodium, potassium, and chloride are mostly in true solution and in ionic forms in the milk, hence can easily diffuse across membranes during ultrafiltration and electro dialysis. Calcium and magnesium, phosphate and citrate are partly in solution and partly in colloidal suspension, depending on the pH of milk. Approximately, 20–30% of diffusible calcium and magnesium are present as free ions and the remainder as salts of citrate and phosphate. As the pH of milk decreases during the manufacture of yogurt and fermented milks, the colloidal form is converted progressively to the ionic form. In addition to the importance of minerals in the stability of casein, the monovalent ions, together with lactose and other low-molecular-weight components, maintain the osmotic pressure at a value iso-osmotic with that of blood. Milk is an important source of dietary calcium and the association with caseins may improve absorption in the GI tract.

Vitamins, Minor Components, and Micro-Nutrients

The concentration of fat-soluble vitamins A, D, E, and K and water-soluble vitamins B and C and other minor constituents is shown in Table 4.10.

Milk contains both fat-soluble and water-soluble vitamins. Low-fat and skim milk will have less fat-soluble vitamins as they get concentrated in the cream fractions during separation. Whole milk is a good source of vitamin A, but the separation process leads to reduced vitamin A in low-fat and skim milk. The FDA regulation requires fortification of low-fat milk and skim milk to restore and to make the vitamin content of low-fat and skim milk equivalent to that of whole milk. Natural vitamin A activity in milk is due to retinol and the pigment β -carotene. Vitamin D is important in bone health and vitamin E is an antioxidant. Vitamin K is present in milk but its dietary nutritional role is minor. Milk and dairy

Table 4.10. Vitamins in Bovine Milk

Vitamins	Per 100 g of Milk
Thiamine (B1)	45 µg
Riboflavin (B2)	175 µg
Niacin	90 µg
Pyridoxine (B6)	50 µg
Pantothenic acid	350 µg
Biotin	3.5 µg
Folic acid	5.5 µg
Vitamin B12	0.45 µg
Vitamin C	2 mg
Vitamin A	40 µg (RE)
Vitamin D	4 (IU)
Vitamin E	100 µg
Vitamin K	5 µg

products can provide substantial amount of retinoids and carotenoids in the diet. One quart of whole milk contains about 36–40% of the RDA for an adult male. Milk is an important source of dietary B vitamins. They are stable to various heating and processing conditions milk is normally subjected to. Riboflavin is vulnerable to light, generating sunlight flavor defects in milk. Ascorbic acid (vitamin C) content of milk is very low and not significant. Most of the vitamin C content of milk is destroyed during pasteurization.

Milk Enzymes

Enzymes in milk occur in various states: (1) as unassociated forms in solution, (2) associated or an integral part of membrane fractions, such as the fat globule membrane or skim milk membrane vesicles, both of which are derived from the plasma membrane of the secretory cell, (3) associated with casein micelles, and (4) as part of the microsomal particles.

Milk contains a large number of enzymes (approximately 60). The partition and distribution of these enzymes is affected by processing and storage conditions of milk. The origin of these enzymes in milk is from cow's udder (synthesized enzymes) or from bacterial enzymes (bacterial source). Several of the enzymes in milk are tested for quality assurance of raw milk and processed milk products. Some of the minor enzymes, such as aldolase, lactate dehydrogenase, arylsulphate, catalase, and *N*-acetyl- β -D-glucosaminidase are associated with somatic cells and thus their presence is related to disease of the mammary gland, particularly mastitis. Enzymes as-

sociated with membrane fractions will occur in both cream and skim milk. Skim milk membrane vesicles may pass on to the whey fraction upon casein curd formation.

Enzymes of known or potential technological significance include plasmin, lipoprotein lipase, alkaline phosphatase, lactoperoxidase, sulphydryl oxidase, *N*-acetyl- β -D-glucosaminidase, catalase, xanthine oxidase, superoxide dismutase, γ -glutamyltransferase, and lactose synthase.

Some enzymes that are important for dairy processing are described below.

Plasmin (Protease). This enzyme hydrolyzes proteins. Limited proteolysis of β -casein by this enzyme is responsible for the presence in milk of large polypeptides derived from this protein, known as the γ -caseins. Activity of this enzyme is also important in cheese ripening and the stability of casein micelles in various products such as UHT milk. The optimal activity of this enzyme is observed at a temperature of 37°C and a pH of 8.0. Nearly, 80% of its proteolytic activity is lost when milk is pasteurized. Microbial-derived proteases are more heat stable than native proteases in milk and they tend to survive even UHT processing. Residual proteolytic activity can affect shelf life of milk and milk products.

Lipoprotein Lipase. The enzyme is present in freshly drawn milk, but the protective effect of the milk fat globule membrane means that significant lipolysis due to lipoprotein lipase is rare. Beneficial effects of its activity include the possible aid in initial digestion and absorption of milk lipids in the intestinal tract and flavor in certain cheeses made from raw milk. However, lipolytic activity also causes a hydrolytic rancid flavor. This enzyme hydrolyzes triglycerides liberating fatty acids and glycerol. The volatile short-chain fatty acids generate undesirable rancid flavors in milk. Normally the substrate (glycerides) is not accessible to the enzyme; however, rapid cooling can dissociate the enzyme from micelles allowing it to attach to the fat globules resulting in "spontaneous lipolysis" and mechanical treatment (e.g., homogenization) of unpasteurized milk can disrupt the fat globule membrane allowing interaction with casein micelles and its associated lipase. Postpasteurization contamination of pasteurized milk with raw milk allows development of rancid flavor during short period of storage. The optimum pH for lipase activity ranges from 8.4 to 9.0, while optimum temperature for enzymatic activity is 37°C. Minerals

such as sodium and magnesium tend to stimulate the lipase activity, while calcium and magnesium show an inhibitory effect.

Alkaline Phosphatase. This enzyme is of commercial importance because of its widespread use as an indicator of pasteurization efficiency. The enzyme's heat-stability profile closely follows that necessary for adequate pasteurization. The time-temperature treatment for pasteurization of milk is based on the destruction of pathogens. Alkaline phosphatase activity is also destroyed by the pasteurization process. Thus, testing for the presence of alkaline phosphatase in pasteurized milk will also test the efficiency of pasteurization in killing pathogens and making the pasteurized milk safe from pathogens. Postpasteurization contamination of heat-treated milk with raw milk can also be detected by positive phosphatase activity in milk. Alkaline phosphatase is distributed throughout milk. Its concentration is higher in the cream fraction. The optimum pH for the action on alkaline phosphatase on p-nitrophenyl-phosphate is 9.5.

Lactoperoxidases. Commercial interest has also been expressed in the use of lactoperoxidase, activated by addition of thiocyanate, as an antibacterial agent. For example, the activity of this enzyme has been used to prevent microbial deterioration of nonrefrigerated unpasteurized milk during collection and storage in developing countries. Its availability through large-scale isolation from whey has also stimulated interest in nondairy application such as dental products. Lactoperoxidase displays optimum activity at pH of 6.0 and is stable over a wide range of pH between 5.0 and 10.0.

FACTORS AFFECTING COMPOSITION, QUALITY, AND SAFETY OF MILK

Milk differs widely in composition. All milks contain the same kind of constituents, but in varying amounts. Milk from individual cows shows greater variation than mixed herd milk. In general, milk fat shows the greatest daily variation, then protein, followed by ash and carbohydrate.

The factors affecting the composition of milk are:

1. *Species*: Each species yield milk of a characteristic composition.
2. *Breed*: In general, breeds producing the largest amounts of milk yield milk of a lower fat percentage.
3. *Individuality*: Each cow tends to yield milk of a composition that is characteristic to that individual.
4. *Interval of milking*: In general, a longer interval is associated with more milk, with a lower fat content.
5. *Completeness of milking*: If a cow is completely milked, the composition is normal, if not, it is usually lower.
6. *Frequency of milking*: Whether a cow is milked 2, 3, or 4 times a day, has no great effect on the fat content.
7. *Irregularity of milking*: Frequent changes in time and interval of milking result in further variability.
8. *Day-to-day milking*: It may show variations for the individual cow.
9. *Diseases and abnormal conditions*: These tend to alter the composition of milk, especially when they result in a fall in yield.
10. *Portion of milking*: Foremilk is low in fat content (less than 1%), while strippings are highest (close to 10%). The other milk constituents are only slightly affected on a fat-free basis.
11. *Stage of lactation*: The first secretion after calving (colostrum) is very different from milk in its composition and general properties.
12. *Yield*: For a single cow, there is a tendency for increased yields to be accompanied by a lower fat content and vice versa.
13. *Feeding*: This has temporary effect only. Overfeeding does not increase the normal flow of milk, but underfeeding has a significant effect.
14. *Season*: The percentages of both fat and SNF show slight, but well-defined variations during the course of the year.
15. *Age*: The fat percentage in milk declines slightly as the cow grows older.
16. *Condition of the cow at calving*: If the cow is in good physical condition when calving, it will yield milk of a higher fat% than it would if its physical condition was poor.
17. *Excitement*: Both yield and composition is liable to transient fluctuation during periods of excitement.
18. *Administration of drugs and or hormones*: Certain drugs may affect temporary change in fat content. Injection or feeding hormones results in an increase of both milk yield and fat content.

Generally, these compositional variations tend to average over in pooled bulk milk used by the dairy processing plants; however, it displays an important seasonal pattern. The seasonal variation includes fat and SNF content. For example, variation in protein and mineral content has an impact on viscosity and gel structure of yogurt and fermented products. Similarly, variation in protein could affect the frothing properties of milk which is important for cappuccino coffee-making in cafes. Seasonal variation in fat could affect the organoleptic properties and texture of the finished products. Low-SNF content can affect the yield and overrun of ice cream. Low protein and calcium contents result in poor viscosity, texture, and yield in yogurt and cheese. In some instance, addition of milk solids (skim milk powder) is necessary to upgrade the formulation of dairy mixes to manufacture quality dairy products.

FUNCTIONAL AND DAIRY-DERIVED INGREDIENTS

Milk and other dairy products contain a wide range of potential functional ingredients and useful precursors to such ingredients.

CALCIUM

Normal milk contains approximately 1.2 mg of calcium per milliliter of milk. Most milk and dairy products can deliver 100% of the RDI of calcium in a reasonable serve of the product. Of the serum phase calcium, most of it is truly soluble and is removed during processing which remove salts and lactose, such as during the preparation of whey protein concentrates and isolates. In rennet-coagulated product such as cheese, the relative proportion of calcium in either serum or micellar phases of milk indicate the retentive ability of calcium. Thus, the level of calcium in dairy products and ingredients vary significantly as the proportion of total solids. The bioavailability of calcium in milk is well known compared to vegetable sources and mineral supplements. Dairy-based food ingredients are excellent vehicle for enrichment of calcium in foods. Dairy sources of calcium provide bone and teeth health, protect against cardiovascular diseases, infectious diseases, colon cancer, and kidney stones. Thus, dairy ingredients represent critical constituent for recommendation in public health nutrition campaigns.

LACTOSE

Lactose along with lactulose, lactitol, and galactooligosaccharides has a useful role as ingredients in processed food manufacture. Intolerance to lactose intake by a high proportion of population has restricted its use. Maldigestion and hence malabsorption of lactose and the associated gastrointestinal symptoms is less with yogurt and fermented milks than in whole milk. The level of lactose in traditional yogurt can alleviate maldigestion as 20–30 g/L are hydrolyzed by the lactic acid bacteria (LAB) during fermentation. However, in current commercial practice, milk solids are usually added to milk before fermentation, thus the resulting fermented yogurt has lactose levels similar to that of milk. Nevertheless, lactose maldigestion is much reduced in humans consuming yogurt. Lactose maldigesters more effectively digest lactose from yogurt than in milk. This can be attributed to the continued activity of the microbial β -galactosidase from the yogurt cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) in the GI tract. Lactose-free and reduced-lactose products are available in most countries include milk, yogurt, and cheese. Lactose reduction is usually achieved through the action of enzyme β -galactosidase through immobilized enzyme technology and chromatographic methods. Hydrolyzed lactose forms a sweet syrup due to increased glucose concentration during hydrolysis. Lactose-hydrolyzed syrups are usually made from dairy permeates and wheys and are used in confectionery and ice cream formulations.

CASEINS

Casein contains a significant source of nonessential amino acids with a high degree of phosphorylation. A key nutritional role of caseins is for stabilization and delivery of nanoclusters of calcium phosphate to the newborn delivered through a digestible polypeptide vesicle. A commercially available food ingredient known as “Recaldent”TM (Cadbury-Schweppes, Australia) contains a casein phosphopeptide class of peptides which is claimed to protect against the demineralization of tooth enamel.

Caseinates are soluble with alkaline treatment and has a random coil structure with low percentage of helix, hence exhibits limited heat gelation and denaturation, but provides high viscosity in solution. Caseinates have high electric charges and several hydrophobic groups. High charges will lead to more

solubility in water and forms an ideal surface-active ingredient with a strong preference to interfaces (fat/water, air/water, build strong flexible membranes (inert to heat), thus potential for developing natural food packaging film and coatings. Caseinates are used in small goods, ice cream and cheese extenders. The high viscosity of caseinates helps develop a range of textures (cuttable to spreadable) in food products with minimal fat separations.

WHEY PROTEINS

Whey protein concentrates (WPC) are made from whey in concentrated and dried forms. There are low-protein-type WPC (35% protein) and high protein WPC (~75% protein). Whey proteins have many S-S bonds in their structure, hence they are globular and strongly folded structure, and are sensitive to heat. Heat unfolds the globular structure of whey proteins and due to S-S bonds linkages; the macroeffect is gelation. Whey proteins account for about 20% of the total protein fraction of milk but are of greater intrinsic nutritional value because of the high relative abundance of both nonessential and essential amino acids, particularly sulfur containing amino acids. Thus, nutritional, physical, and physiological functional activities are embodied in whole whey protein concentrates, which have led to their growing application in health food products, such as snacks, bars, and beverages.

WHEY PROTEIN DENATURATION, AGGREGATION, AND APPLICATIONS

The denaturation of whey proteins can be described by formal reaction kinetics which enables us to consider the many different dependencies. There are two ranges with different activation energies: one at temperature below 90°C where unfolding is the rate limiting reaction and the other over 90°C with the aggregation reactions as determining steps. Increased WP concentration results in an increased denaturation degree. The influence of the lactose concentration showed a retardation of the reaction with higher concentration, especially at temperature below 90°C. UHT pressure treatment can also be used for the denaturation of whey proteins. If denaturation degree of β -lactoglobulin (approximately 0.4% of whey protein) is higher than 90% in heat-treated milks, properties such as firmness water-binding capacity, syneresis,

and viscosity will be improved in fermented milk products by aggregating proteins. At higher temperatures, the aggregate densities increase in addition to the particle size. High voluminities are the result of heating at temperatures below 90°C where unfolding is the rate determining reaction. Calcium content influence aggregate size, increasing aggregate size with higher calcium contents. High level of the lactose content inhibits denaturation. A creamy smooth mouthfeel can be produced at low-lactose concentrations and at high-denaturation degrees. A few examples of this utilization of particulated whey protein include the production of soft cheese, and ice cream. In soft cheese, yield increases and the sensory properties improve. To activate a high yield of particulated whey proteins, the whey needs to be concentrated and diafiltered to reduce the lactose content, which is required in order to obtain a high-denaturation degree. The application of microparticulated whey protein in ice cream improves the structure and mouthfeel. The whipping time of fat-reduced cream (20% fat) can be decreased from 800 to 370 seconds if 2% microparticulated whey proteins are added instead of 2% whey concentrate.

MILK PROTEIN HYDROLYSATES

Milk protein hydrolysates (MPH) produced with controlled enzyme reaction modify the functional performance of whey proteins. When proteins are hydrolyzed, the primary chains are reduced in size. The hydrolysis process can both be performed by means of chemicals (in acidic or alkaline environment) or with the catalytic aid of proteolytic enzymes. Hydrolyzing protein enhances their whipping ability and produce stable foams in products such as marshmallows, mousse, nougat, and confections. MPH can produce stable aerated foods in the presence of high levels of fat, carbohydrate, and protein (e.g., rich and light and fluffy chocolate and fruit mousses). One important reason for this marked foaming ability is the fact that the overrun is limited by the availability of foaming ingredients. When large amphiphilic polymers are hydrolyzed, simply more whipping agent is available. Small molecules will diffuse faster to interfaces than large ones. In addition, the composition of the fragment is very important. If the distribution of hydrophobic and hydrophilic patches is not well defined over the primary chain, then the foaming properties will be reduced. Finally, also the flexibility of the chain strongly influence the adsorption behavior of the hydrolysate fragments and

their stabilizing ability. The disadvantage of proteins when used as whipping agents is the sensitivity of fat. Fat particles and low-molecular-weight emulsifier are strong antifoaming agents. Protein hydrolysates are also used as yogurt stabilizers and to reduce fermentation time, reduce starter culture inoculum size, control postacidification enhance probiotic organism numbers, and improve texture.

α -LACTALBUMIN AND β -LACTOGLOBULIN

α -Lactalbumin is a calcium metalloprotein involved in the regulation of lactose synthesis (i.e., UDP-galactose *N*-acetyl glucosamine β -1–4 galactosyl transferase-I) and therefore in regulation of the production of lactose. Unlike the caseins, the presence of one calcium ion per α -lactalbumin molecule serves to stabilize the structure and does not transport significant levels of calcium per se. α -Lactalbumin is present at higher level in human than in bovine milk and therefore the fortification of infant formulae with respect to this protein is thought to improve its comparability to human milk. α -Lactalbumin shows apoptotic effects on transformed cells and exerts its activity through the penetration of phospholipid bilayers. The properties of β -lactoglobulin have been intensively studied including crystal and NMR structures that have revealed an open β -barrel enclosing a hydrophobic cleft and a single 3-turn α -helix. The dimeric structure present under milk-like conditions dissociates under acid conditions (around pH 2.0), and between pH 3.5 and 6.5. As a member of the lipocalcin family, biological functions of β -lactoglobulin have been associated with the binding and carrier functions of small hydrophobic molecules in the hydrophobic cleft.

LACTOFERRIN AND LACTOPEROXIDASE

In cheese whey, the major whey proteins α -lactalbumin and β -lactoglobulin are negatively charged, whereas lactoferrin (Lf) and lactoperoxidase (Lp) are positively charged. This property affords ready separation of Lp and Lf from the major protein by ion-exchange methods. Lf is a globular protein found in most mammalian secretions including milk. Bovine Lf is substantially homologous with human Lf. It binds iron (Fe) and is structurally similar to serotransferrin, the plasma iron transport protein, but Lf has a much higher efficiency for iron (250 fold). Bovine milk contains about 20–200 mg/L

of Lf while human milk contains approximately 2 g/L of Lf. Bovine colostrum, particularly during the first days of lactation, contains about 2–5 g/L of Lf. The contents of Lf in cheese whey varies from 5 to 150 mg/L. New-generation infant formulae supplemented with bovine Lf may represent the leading application of the protein, with the intention of stimulating the high content of Lf in human milk. Lf is thought to provide nonimmune protection to the neonate against bacterial and viral infections through its antimicrobial activity. Bovine lactoferrin inhibits bacterial growth by its ability to sequester iron and also permeabilize bacterial cell walls by binding to cell wall lipopolysaccharides and porins through its N-terminus.

The protein is active against a range of pathogenic and nonpathogenic organisms, including but not limited to *Aerobacter*, *Bacillus*, *Candida*, *Clostridium*, *Pseudomonas*, *Salmonella*, *Shigella*, *Streptococcus*, and *Staphylococcus*. Lf has been shown to be effective in reducing enteric infections in children by inhibiting the growth of some of these bacterial strains and in enhancing the growth of “friendly” bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium*. There is some evidence that Lf enhances the absorption of iron in milk-fed infant and limits food intolerance, intestinal infections, diarrhea, and possibly colic. Lf may also have a role in the formulation of cosmetics as a natural antibacterial agent. Lf plays a role in reducing the effects of skin ageing by inactivation free radicals. The strong inhibition of *Streptococcus* mutants by Lf provides the basis for the use of this protein (either alone or in combination with the “lactoperoxidase system”) in oral health care products such as toothpaste and mouth wash.

Lf is a major antibacterial enzyme in colostrum (11–45 mg/L). It is a greenish-brown hemoprotein found in bovine milk (10–50 mg/L) and cheese whey (5–30 mg/L), and also in other mammalian secretions, such as saliva and tears. Lp is from the heme peroxidase family of proteins which catalyze the peroxidation of acceptor molecules such as thiocyanate. In the presence of lactoperoxidase, the thiocyanate ion reacts with hydrogen peroxide to form oxythiocyanate ion which is a potent antimicrobial agent. Thus, a combination of thiocyanate (or other acceptor molecule), hydrogen peroxide, and lactoperoxidase constitutes the “lactoperoxidase system” and represents an effective antimicrobial system. The lactoperoxidase system is active against a wide variety of Gram-positive and Gram-negative organisms,

including *Campylobacter*, *Escherichia*, *Lactobacillus*, *Listeria*, *Salmonella*, and *Staphylococcus*. The antimicrobial activity of the lactoperoxidase system forms the basis for applications of lactoperoxidase. These include, but are not limited to, enhancement of the shelf life of perishable foods (e.g., refrigerated and nonrefrigerated milk, infant formulas) and as an antibacterial agent in oral health care products (e.g., tooth paste and mouth wash).

IMMUNOGLOBULIN

Immunoglobulins (Ig) are glycoproteins each comprising two glycosylated “heavy” and two “light” chain subdomains designed to bind antigens and elicit host defense processes, and thereby offer passive immunity to either calves or other potential consumers. Immunoglobulins are present at about 40–200 mg/mL and 0.7–1.0 mg/mL in colostrum and milk respectively, mainly as IgG 1. Immunoglobulins are intrinsically heterogeneous due to their physiological function, particularly in the primary sequence responsible for antigen binding, and are nutritionally valuable proteins because of their richness in sulfur-containing amino acids. Colostrum, which is the richest source of bovine immunoglobulins, is under intense development as a food ingredient, targeting lucrative niche markets, such as sports nutrition, which is large and growing rapidly, particularly in North America.

Bovine immunoglobulins are utilized either for their polyclonal-binding activity raised against specific antigens through hyperimmunization or as nonspecific polyclonal antibodies. Bovine immunoglobulins, expressed in colostrum, have been raised against both bovine and human pathogens through hyperimmunization methods, with intended applications for passive immunization of the neonate or human consumer. Nonspecific polyclonal antibodies in bovine colostrum appear to offer protection against human forms of selected pathogens, such as *Cryptosporidium parvum*.

In spite of the low-relative abundance of immunoglobulins in milk, hyperimmune milk products are also in the market place and have been demonstrated to exert benefits including the treatment of protozoal gastrointestinal disorders, immune suppression, rheumatoid arthritis, prevention of suppression of T-lymphocytes function, and passive immunization. The reported protection of orally ingested bovine colostrum concentrate through the stomach to the lower bowel and associated protective benefit

against infection have elicited speculation regarding the application of this product as a functional food ingredient for at-risk populations, including developing nations.

BIOACTIVE PEPTIDES

Some milk peptides released during the digestive process exhibit biological activities over and above their intrinsic nutritional value. Hydrolysates of milk proteins have been produced using proteolytic enzymes and fractionated in order to study their in vitro bioactivities. More recently, yogurt and cheese fermentation processes have also been optimized to release bioactive peptides.

Selected milk-derived peptides are also known to exhibit bi-functionality, for example, the casein phosphopeptide class of peptides have been shown to exert immunostimulatory and anticancer properties. Bioactivities of milk-derived peptides, including mineral-binding, opioid agonist and antagonist, immunostimulation, cell growth modulatory, ACE inhibitory, antithrombotic, antioxidant, and antimicrobial properties have been reported. Emerging clinical evidence and the commercial development of the bioactives of milk-derived peptides indicates that dairy products represent the leading edge of commercial development of bioactive peptides as functional ingredients.

Fermentation has been used to produce milks containing bioactive peptides that lower blood pressure in hypertensive subjects. The microorganism *Lactobacillus helveticus* is normally used in fermentation as it possesses strong proteolytic activity compared to most lactobacilli. Examples of products are “Calpis Sour Milk” (Calpis, Japan), “Evolus” (Valio, Finland) and another Finnish product, “Festivo cheese.” Peptides with clinically substantial bioactivities to date include Val-Pro-Pro, Ile-Pro-Pro, and Val-Tyr, all of which exhibited antihypertensive activity linked to ACE inhibition. Yogurts and sour milks represent particularly attractive vehicles for both production and the delivery of bioactive dairy peptides, whereas cheese as a delivery vehicle may be more problematic because of the progressive hydrolysis of proteins occurring during maturation. A number of whey, casein, and dairy protein-derived peptides, and hydrolysates are in the market place. For example, the Danisco “Biozate” range of whey protein-based hydrolysates has been patented for their antihypertensive properties and digestibility.

SPECIFIC LIPIDS

In addition to the saturated fatty acids (SFA), trans-fatty acids (TFA), and cholesterol fraction, bovine milk fat contains multiple lipids with important bioactivities. These include conjugated linoleic acid (CLA), sphingolipid, butyric acid, n3- and n6-PUFA, branched fatty acids, and ether lipids. Levels of CLA are correlated with levels of TFA, but inversely correlated with levels of SFA. Mostly, the adverse health effects of FFA and SFA have been ascribed to their role in the promotion of atherosclerosis, whereas CLA is antiatherogenic and CLA together with the other nominated favorable lipids are reported to exert anticancer properties. On the basis of current knowledge, CLA is likely to play an important role in improving the nutritional acceptability of milk fat and dairy products in the future.

Improving the healthiness of milk fat can be achieved through the manipulation of the ratios of SFA to USFA through cow nutrition. Milk fat spread products with elevated ratios of unsaturated to saturated fatty acids are promoted for both health and associated spreadable characteristics. There is a growing opinion that synergistic effects of multiple components of whole milk may outweigh the negative effects of SFA and TFA. These synergies refer to activities of calcium and bioactive peptides which are directly protective against hypertension, together with the vitamins and vitamin transport factors present in milk, which are protective against hypertension through the lowering of homocysteine levels.

GALACTOOLIGOSACCHARIDES, LACTULOSE, LACTITOL, AND LACTOSUCROSE

Galactose–oligosaccharide (GOCS), lactitol, lactosucrose, and lactulose are all derived from lactose, and all have been shown to possess prebiotic properties (e.g., exhibit bifidogenic effects). A number of other nondigestible carbohydrates (e.g., fructooligosaccharides, inulin, and resistant starch) also have prebiotic properties but these are not derived from milk. However, these nondairy ingredients are commonly used in the production of some yogurt. Lactulose has been recognized as a bifidogenic factor enhancing the levels of bifidobacteria in the lower colon area of the GI tract of humans. Lactosucrose (β -D-galactopyranosyl-1-(1-4)- α -D-glucopyranosyl β -D-fructofuranoside) is recog-

nized by the Japanese FOSHU regulatory system as a bifidogenic factor and has been shown to increase fecal bifidobacteria in long-term human trials. It also has been shown to be possibly effective in beneficially modifying fecal flora in patients with chronic inflammatory bowel disease.

Lactitol is the sugar alcohol of lactose and is produced by catalytic hydrogenation. A bifidogenic effect of lactitol has been recorded. At 20 g/day in human diets, lactitol significantly reduced the activity of procarcinogenic enzymes and aromatic compounds in the colon. Health effects claimed for prebiotics are:

1. Enhanced mineral uptake (e.g., calcium, magnesium, iron).
2. Reduction of serum lipids (and possibly cholesterol) via acetate production and reduced recycling of bile salts.
3. Reduced risk of intestinal infections by providing alternative soluble receptor sites for pathogenic organisms, thus altering colonization resistance and competitive exclusion.
4. Reduction in risk factors for colon cancer by reducing protein metabolism in the colon, reducing levels of genotoxic enzymes, reducing secondary bile salt production, and increased production of short-chain fatty acids, predominantly butyrate.

Generally, the physiological properties of prebiotics are related to the chemical structure and molecular size of the prebiotic compounds. These will control the extent of digestion of the compound during passage through the stomach and small intestine. The molecular structure of the prebiotic will also determine which microbial species are able to utilize it as an energy source in the bowel. The physicochemical properties of the prebiotic compound are also determined by its molecular size. They may affect taste, sweetness, mouthfeel, viscosity, solubility, hygroscopicity, color reactions on heating, freezing point, osmolality, and crystal formation. Many prebiotics will act as low cariogenic, low calorific value, low sweetness compounds, with a potential to reduce the glycemic index of the food. It is well established that oligosaccharides, dietary fibers resistant starches, and inulin are safe in high doses. However, intake exceeding 15–20 g daily in adult human of shorter chain oligosaccharides such as fructo- and galactooligosaccharides can lead to flatulence abdominal discomfort, and cramping. Excess lactulose consumption can result in diarrhea. Recommended

effective doses of oligosaccharides in adult human usually range between 10 and 20 g/day.

PHYSICAL CHARACTERISTICS OF MILK

PHYSICAL STRUCTURE

Milk is extremely complex biological fluid with scores of nutrients contained in a fluid with characteristics of three physical phases: an emulsion, a colloidal dispersion, and a solution. Various interactive forces among the constituents of milk determine the technological behavior of milk. Milks are biological fluids of exceptional complexity containing thousands of compounds. These are located in several compartments directed there by the biological and physiological forces acting during milk synthesis, secretion, and thereafter. These compartments in bovine milk are altered by processing. Most of the milk is processed, that is, pumped, agitated, pooled, cooled, clarified (centrifuged) to remove cells, fat content standardized (centrifugation), pasteurized, and homogenized to reduce the size of the fat globules.

Milk lipids present as an "oil-in-water" type emulsion can be broken by low-speed centrifugation and the milk separates into lipid and aqueous phases, each with a characteristic composition. The colloidal phase contains casein micelles, calcium phosphates, and globular proteins. Whey proteins are in colloidal solution and the casein is in colloidal suspension. With ultracentrifugation, the casein micelle precipitates the supernatant remaining after the process has the characteristic of a true solution. Lactose, vitamins, acids, enzymes, and some inorganic salts are present as true solutions. The physical equilibrium of milk that exists between colloidal dispersion and salts is destabilized by factors such as (a) addition of polyvalent insoluble salts, (b) concentration of serum solids, (c) changes in pH, (d) heat treatment, and (e) addition of precipitant such as alcohol. These factors can change the structure of milk and its physical equilibrium. For example, in the manufacture of fermented milks, the reduction of pH can cause the physical equilibrium to destabilize and forms a gel. Casein and the interacted whey proteins coagulate at the isoelectric point at pH 4.6, forming a gelled structure.

PHYSICAL PROPERTIES

The physical properties of milk are of great importance to the dairy technologist, as they will affect

most of the unit operations during processing. These include fluid flow, mixing and churning, emulsification and homogenization, as well as heat transfer processes such as pasteurization, sterilization, evaporation, dehydration, chilling, and freezing. Some of the rheological properties are also used for assessing and monitoring the quality of products, such as yogurts, cream, butter, and cheese.

Electrical Conductivity

This is defined as a measure of the electrical resistance of the solution in reciprocal ohms (mhos). It is used to assess the total ionic content of milk. The greater contributors to conductivity are the sodium, potassium, and chloride ions. Because the amounts of sodium and chloride increase with mastitis, measurements of conductivity in bovine milk are employed to screen for clinical cases of the disease. Most dairy products are poor conductors of electricity. The specific electrical conductivity of bovine milk at 25°C ranges from 0.004 to 0.005 mho/cm. It might be expected that increasing the concentration of milk solids would increase the specific conductance, but the relationship is not so linear. The conductance of concentrated skim milk was shown to increase to a maximum value of about 0.0078 mho/cm at 28% total solids, after which it decreased. This is because of the extremely complex salt-balance between the colloidal and soluble phases. The presence of fat tends to decrease the specific conductance, the specific conductance of milk fat being less than 10^{16} mho/cm. Whey and permeate from ultrafiltration have higher conductivity than skim milk. Conductivity of milk may be used to detect any residual cleaning agents such as sanitizers in the milk.

The development of acidity occurring during much fermentation has also been observed to increase conductivity due to conversion of calcium and magnesium to ionic forms. Hence, electrical conductance may be used as a process monitoring index during fermentation of yogurt and other fermented products. During demineralization of whey, electrical conductance can decrease due to loss of ionic minerals. For example, the decalcification of sweet whey at pH 3.0 decreased the conductivity and resulted in 94% demineralization. The conductivity of milk and dairy products will be important in ohmic heat processing.

OXIDATION-REDUCTION POTENTIAL

The oxidation-reduction (Eh) potential of milk is expressed in volts. In milk, Eh depends on factors such as

dissolved oxygen, ascorbic acid, riboflavin, cystine–cysteine contents, and pH. Fresh bovine milk has Eh value between +0.2 and +0.3 V at 30°C. During fermentation of milk, aerobic bacteria use up the dissolved oxygen and reduce the oxygen tension in the milk and this favors the growth of anaerobic bacteria. Microbial quality of milk is sometimes assessed using methylene blue reduction test which is based on this principle.

The increase in reducing capacity of yogurt mix after treatment is important in promoting the growth of yogurt bacteria which are microaerophilic in nature.

The dissolved oxygen in dairy products such as yogurt during its shelf life is important for the viability of probiotic bacteria such as *Bifidobacterium bifidum*. It has been shown that oxygen can gain entry into yogurt through the packaging materials and hence can increase the dissolved oxygen content and bifidobacteria do not survive well in oxygenic conditions. The ascorbic acid oxidation in stored milk leads to the formation of singlet oxygen, which can promote lipid peroxidation leading to spoilage of milk. When milk is exposed to UV light, riboflavin in milk is activated and starts a series of reactions including photooxidation of methionine and this leads to flavor defect. Packaging materials blue in color or with a layer that prevents ingress of sunlight prevent these reactions occurring in the milk.

Rheological Properties

Rheology is the study of deformation of materials, subjected to applied forces. A distinction is usually made between fluids and solids. Fluids will flow under the influence of forces, whereas solids will stretch, buckle, or break.

Viscosity. Milk, skim milk, cheese whey, and whey permeate, all can be regarded as dilute solutions and are usually considered to be Newtonian fluids. The viscosity of milk is around 2.2–2.5 mPa.s at 20°C, and this depends on the metabolism and state of nutrition of the individual cow. The non-Newtonian behavior is only of consequence as it affects the drainage of vessels that have contained milk. When milk is poured from a bottle, a thin film may remain and this is only in part due to the adhesion of milk solids to the container walls; the increased viscosity at low shear rates associated with drainage also makes its contribution. The viscosity of all fluids is temperature dependent. Viscosity of milk and dairy products depends on the temperature and on concentration and state of casein

micelles and fat globules. Representative values at 20°C are: whole milk, 1.9 cP; skim milk, 1.5 cP; and whey, 1.2 cP. Homogenization breaks up the fat globule into many tiny globules hence increases viscosity of milk and cream. The viscosity of milk and cream provides “richness” perception to the consumer.

The casein micelles of milk contribute more to the viscosity of milk than any other milk constituents. Hydration of protein can also cause an increase in viscosity. The viscosity of skim milk decreases on heating to 62°C after which it increases apparently due to changes in protein hydration. An increase in temperature causes a marked reduction in viscosity. For example, at 20°C, milk is about half as viscous as at 0°C, and at 40°C, it is approximately 1/3 of the value at 0°C.

The viscosity of solutions increases as the concentration increases in a nonlinear fashion. At high concentrations, small additional changes in the concentration will lead to rapid changes in viscosity. This may result in decreased flow rate, drop in pressure, reduced turbulence, and fouling. In dairy fluids concentration processing such as evaporation, reverse osmosis and UF, the final concentration may well be limited by viscosity consideration. The viscosity of full cream evaporated milk will depend upon the degree of forewarming, homogenization conditions, type of stabilizer used and the extent of the final in-container heat treatment. Viscosity is one of the main factors which limit the extent of concentration for UF and RO processing of dairy fluids. The protein fractions make the main contribution to the viscosity. Freshly separated cream has a fairly low viscosity. The market cream is then standardized to the desired fat content, heat treated, homogenized, cooled, and packaged. All of these factors can significantly affect the viscosity of the final product, and each cream needs to be treated differently to obtain the best quality product.

SURFACE PROPERTIES

Surface tension is defined as the work required to increase the surface area of a solution and is expressed as dynes/cm. Surface properties are involved in adsorption and formation and stability of emulsions. The surface properties are relevant to creaming, fat globule membrane function, foaming, and emulsifier use in dairy products. The surface tension of milk approximates 70% of that of water (72 dynes/cm). The surface tension of cow's whole milk ranges from 50 to 52 dynes/cm and for skim milk,

55 to 60 dynes/cm at 20°C. For cream, it is approximately 46–47 dynes/cm. Casein, along with its proteolytic products proteose-peptones, is largely responsible for the surface tension. Fat reduces surface tension by a physical effect. Lactose and most of the salts tend to raise it when they are present in a true solution.

This property is used to follow the changes in surface-active components during milk processing, to follow release of fatty acids during lipolysis, and as a measure of the foaming tendency of milk. Fatty acids and their salts and monoacylglycerols formed as a result of lipolysis are surface active and reduce surface tension. However, the method is not applied routinely for the assessment of lipolysis, because the short-chained acids responsible for the flavor designated as hydrolytic rancidity are water soluble and do not affect surface tension. The interfacial tension between the fat-soluble and the aqueous medium, of considerable potential importance in emulsion stability and access by lipolytic enzymes, cannot be determined directly.

Temperature affects the degree of surface tension, processing treatments such as heating; sterilization, homogenization, and shear tend to increase surface tension. However, homogenization of imperfectly pasteurized milk or contamination of homogenized and pasteurized milk with raw milk causes partial hydrolysis of milk fat resulting in low surface tension, bitter flavor, and rancidity of milk.

FOAMING

When milk is aerated it forms foams. Foam is a structure where air cells are incorporated and contained within a protein film matrix. The formation of stable foam depends on the lowering of surface tension which allows the spreading of the surface-active components into thin film provided the film are sufficiently elastic and stable to prevent the coalescence of the air cells created. Stable foam is thus formed when the surface tension of the liquid is not great enough to withdraw the film from the gas cells and when the stabilizing agent has great internal viscosity.

Foaming properties affect handling of milk and incorporation of dairy ingredients. For dairy mixes that need overrun to create texture, forming stable foam is important. Foaming in pipelines will reduce the efficiency of milk heat treatment. For example, during heat treatment of yogurt mix, antifoaming agents are used as processing aid to control foam developed in pipelines. Foam control is necessary for effective

heat treatment as foam development will reduce the effective destruction of pathogenic bacteria during pasteurization. In foaming, the foams constitute air cells enveloped by protein film. The development of foam may be reduced when there is less protein in the milk. Because of seasonal variation in the milk composition, and when the total solids contents is low in milk (with less protein content), frothing of milk in beverages such as cappuccino coffee, will be affected.

Thermal Properties

Heat transfer plays an important role in many dairy processing operations, and in most cases, it is desirable to maximize the rate of heat transfer. This offers economic advantages and generally results in a better quality product. When milk is heated it expands in volume, the coefficient of thermal expansion of milk with standard composition is approximately $0.335 \text{ cm}^3/\text{kg}/^\circ\text{C}$ at a temperature range of 5–40°C. The specific heat is defined as the amount of heat required to raise a unit mass of heat through a unit temperature rise. It is nearly similar to heat capacity of water (4.18 kJ/kg/K) and is fairly constant over the range of 0–100°C. The different components in foods have different specific heat values, so it should be possible to estimate the specific heat of a food from knowledge of its composition. Water has the greatest influence on the specific heat. The specific heat values are used to determine the amount of heat or refrigeration required to process milk.

Thermal conductivity is a means of the rate of heat transfer through a material when conduction is the controlling mechanism. It is defined as the steady-state of heat transfer through an area of 1 m^2 when temperature driving force of 1 K is maintained over a distance of 1 m . Thermal conductivity determines how fast milk is cooled or heated. Thermal conductivity changes with temperature, for a variety of products. Thermal conductivity increases as the temperature increases; however, as milk product becomes more concentrated, their thermal conductivity decreases. Thermal conductivity of proteins, carbohydrates, fat, milk solids, and water at 30°C is 0.20, 0.245, 0.18, 0.26, and $0.573 \text{ kcal/m/h}/^\circ\text{C}$, respectively. The thermal conductivity for milk at 37°C is 193 J/m/s/K and 223 J/m/s/K at 80°C. Thermal conductivity decreases markedly with increase in either fat or total solids.

Thermal processing of milk (e.g., pasteurization and sterilization) is frequently used to extend shelf

life of milk. The effect of heat on milk could alter their textural properties. For example, when yogurt mix is heated to 60–65°C, β -lactoglobulin molecules begin to uncoil themselves and interact with κ -casein forming disulfid linkages. In the manufacture of yogurts, heat treatment is beneficial in denaturing the whey protein and hence increasing water holding capacity and in reducing syneresis of the coagulum. Non-heat-treated yogurt mix or insufficient heat treatment of yogurt mix leads to syneresis in yogurt during storage. When syneresis occurs in the yogurt, this leads to expulsion of water from the yogurt gel on the surface and when a yogurt container is opened, consumers could mistake this for spoilage of yogurt. High heat treatment could initiate Maillard reactions between amino groups of amino acid lysine and the carbonyl group of lactose to form brown melanoidin-pigmented polymers.

The heat stability of milk is mainly determined by the make up of protein as well as the relative concentration of various salts present in colloidal and ionic states. The pH plays a critical role in determining the heat stability of milk. The pH affects both the molecular disassociation of casein components and formation of aggregated protein complexes through protein–protein interactions.

Colligative Properties

Osmolality and Osmotic Pressure. Osmolality is a measure of the total number of dissolved particles in a given volume of solution given in osmol/KJ. Osmolality is one of the colligative properties (dependent on the number of dissolved particles, not their properties) of milk along with freezing point and boiling points. For a given weight, the smaller the molecules the higher will be the osmotic pressure.

The osmotic pressure of milk is quite constant being equal to the osmotic pressure of blood. As a result, the variation in the dissolved substances in normal milk, primarily lactose is small. The total concentration of dissolved materials is responsible for osmolality. Osmolality is proportional to the freezing point of milk. The osmolality of formulations are carefully controlled to resemble that in human milk. The potential renal solute load is calculated from the contents of sodium, chloride, potassium, and calcium. The potential renal solute load if bovine milks is too high for them to be used as the sole food for young infants. They may exceed the renal threshold in infants and may develop kidney problems later in life.

Freezing Point. The freezing point of milk is lower than that of pure water due to the dissolved components such as lactose and soluble salts. Lactose, potassium, sodium, and chloride are the principal milk constituents responsible for 75–80% of the entire freezing point depression. As with osmolality, the freezing point is stable and hence considered as a fairly constant property of milk. The freezing point is measured as a routine test to determine whether bovine milk has been diluted with water and is employed as a legal standard. A cryoscope is used for this purpose. Adulterated milk will show increased freezing point due to lower molal concentration of lactose and salts. Milk with no added water freezes at -0.540°C . During fermentation, when lactose is converted to lactic acid, a significant lowering in freezing point depression occurs. The freezing point of cream, skim milk, and whey are identical with that of milk from which they are prepared. Therefore, the freezing point test does not detect the addition of skim milk or removal of fat from milk samples. If milk adulterated with water is soured it will result in the lowering of the freezing point depression due to increase in the amount of soluble molecules. Sugar in an ice cream mix helps to decrease the freezing point and hence helps obtain smaller ice crystals and a chewy texture.

Boiling Point. The boiling point of milk is higher than that of pure water due to dissolved components. The milk constituents in true solution are mainly responsible for the elevation of boiling point at a temperature greater than 100°C . The boiling point of milk is 100.17°C . For detection of added water, determination of freezing point is more accurate and convenient.

Density. Milk density at 20°C ranges from 1.027 to 1.033 with an average of 1.030 g/cm^3 . Hence the weight of 1 liter of milk would range from 1.027 to 1.033 kg. The density of milk is a useful parameter to convert volumetric measurement to gravimetric measurement and vice versa. Temperature affects density because milk expands when heated and so it becomes less dense as temperature rises. Density, fat content, and SNF fraction of milk (includes protein, lactose, and salt components of milk) are related and it follows that if two are known the third can be calculated.

The relationship is given by the empirical formula:

$$\text{SNF} = 0.22 \text{ fat}\% + 0.25 \text{ density } (D) + 0.72$$

$$D = (\text{Corrected density hydrometer reading} - 1) \times 1,000$$

Table 4.11. Effects of Heat on Milk

Observed changes	Effect on Milk
Removal of soluble gases	Change in titratable acidity
Change of calcium status (soluble to micellar)	Alter pH and free calcium activity
Modification of lactose (lactulose)	Change in titratable acidity
Denaturation of whey proteins	Change in texture, viscosity
Formation of free sulphydryl groups	Development of cooked fl vors
Drop in redox potential	Reduced auto-oxidation of fats, alter heat stability
Maillard browning	Loss of nutritional value, characteristic cooked fl vor, alter heat stability
Dissociation/aggregation of casein micelles	Alter heat stability, change in viscosity, and texture
Formation of lactones and methyl ketones from lipids	Characteristic rancid fl vors
Degradation of vitamins	Loss of vitamin activity and loss of nutritional value
Alter fat globular membrane	Reduced rate auto-oxidation of fats, alter heat stability

An increased fat content or reduced SNF will lower the density of milk. Percent total solids can be calculated as follows:

$$\% \text{ TS} = \% \text{ SNF} + \% \text{ fat}$$

Specific Gravity. It is the ratio of the mass of a solution or a substance to the mass of a similar volume of water. Addition of sugar and milk solids such as in the case of dairy mixes for yogurt and ice cream will exhibit greater specific gravity. For example, the specific gravity of ice cream mix is 1.0544–1.1232, while that of fresh whole milk lies in the range of 1.030–1.035, with an average of 1.032. As milk fat is the lightest constituent, increase in percent fat will lower the specific gravity of milk or dairy mixes. Specific gravity is useful in assessing nonfat milk and the addition of water to milk which lowers specific gravity. The dairy industry employs a special hydrometer, the lactometer, to determine specific gravity and total solids. Corrections are required for milk temperature which differs from 20°C.

EFFECTS OF HEAT ON MILK

The primary aims of heat treatment of milk are to make it safe for human consumption and to lengthen its storage life by destroying microbial contaminants. In addition, to these aims, heat treatment is applied to impart other desirable properties such as fl vor, color, or viscosity. A description of some of the changes that heating can cause in milk, and the effects that these changes create in the properties of milk are shown in Table 4.11.

HEAT STABILITY OF MILK

The heat stability time (HCT) is defined as the time taken for milk to coagulate while being agitated in a sealed tube at a set temperature (usually between 120 and 140°C). A high HCT indicates high heat stability. Although it correlates poorly with commercial practice as different conditions are used and the “end point” for the test is the failure of the milk.

Milk pH and Heat Stability

Heat stability of milk has been shown to be highly dependent on pH. The pH of milk varies throughout the year. In Scotland, a minimum pH was observed in April and a maximum pH in June. Determination of the heat stability (by HCT methods) showed the milk was least stable in June but most stable in October. The natural pH of the milk coincided with the local maximum HCT versus pH profile except in May and June when the natural pH coincided with the local minimum. It was found that heat stability of recombined concentrated milk (18% MSNF, 8% fat) was generally poor from February through until October. Type A milks have a complex pattern of heat stability with a minimum HCT of less than 5 minutes at pH 6.4, rising to a maximum of more than 20 minutes at pH 6.7, a local minimum of about 10 minutes at pH 6.9, and then the stability rises again to be near 20 minutes at pH 7.2. Type B milks show a simpler pattern having a minimum HCT at pH 6.4, then steadily rising with increasing pH to reach a maximum at pH 7.2.

Milk Mineral and Heat Stability

Calcium and phosphate have been found to play a major role in the heat stability of milk. Their roles are complex because of their distribution between the colloidal (or micellar) and serum phases of milk. Within the serum phase there is a further partition between free ionic forms of the salts and salts complexed with other agents. Generally, addition of calcium salts to milk causes an increase in serum calcium ions, an increase in calcium in the colloidal phase, a decrease in pH and a decrease in heat stability. Alteration of calcium concentration by the addition of phosphates or citrates generally causes an increase in heat stability. The mineral content of concentrated milk also influence its heat stability. Reduction of the mineral content of concentrated milk by dialysis of the milk before concentration or concentration of the milk by UF rather than evaporation resulted in increased heat stability in the concentrate determined by HCT method. De-mineralization of milk by ion-exchange resins improved heat stability of milk which had been concentrated by evaporation. It has been found that increasing the levels of protein alone caused little change while the effect of increasing the mineral components alone was similar to the effect seen on increasing the total solids. Higher levels of κ -casein were dissociated on heating milk with increased total solids or minerals, but not with increased proteins. Addition of calcium to recombined concentrated milk (18% MSNF, 8% fat) caused a complete loss of stability (determined as excessive viscosity after heat treatment) while addition of phosphate caused a decrease in viscosity of the heated concentrate (indicating increased stability) and a shift in the pH at which the lowest viscosity was recorded to slightly more acid values. It was also found that there is a link between a seasonal increase in both total and colloidal calcium and a seasonal decrease in the heat stability of concentrated milk.

Milk Protein and Heat Stability

In concentrated milks addition of either whey protein or purified β -lactoglobulin has a detrimental effect on heat stability of concentrated milk. Addition of κ -casein to milk increased the stability of concentrates produced from adjusted milk (adjustment of casein components of milk protein). Modification of the κ -casein by hydrolysis causes loss of heat stability.

Effect of Genetic Variation on Heat Stability

A number of genetic variants of milk proteins and their effects on properties of milk have been reported. The effect of A and B genetic variants of β -lactoglobulin and κ -casein on heat stability have been studied. It was found that milk from κ -casein B cows was most stable while milk from κ -casein A cows the least stable. It was also reported that milks containing the AA variants of β -lactoglobulin were more stable than milks containing the BB variant.

Effect of Glycosylation of Proteins

A number of reports have been made relating to the glycosylation of milk protein and functional properties. The particular protein usually investigated is κ -casein. The oligosaccharides of κ -casein are linked to the protein via an O-glycosidic linkage between the N-acetylgalactosamine moiety of the oligosaccharide and a serine (Ser) or threonine (Thr) residue of the protein. Recent studies have shown that milk (with micelles) with a higher ratio of NeuAc(N-acetylneuraminic acid) are less heat stable, evaluated by HCT method. These micelles were also larger and had a lower level of κ -casein as a proportion of total caseins than other fractions of the milk.

Relationship Between Urea Levels in Milk and Heat Stability

Significant correlation between heat coagulation times (at natural pH) and urea content has been reported. The heat stability of milk is increased by addition of urea and lowered by removal of urea by degradation with urease. It has been suggested that seasonal problem with the heat stability of milk powders may be due to variation in the urea levels. The loss of heat stability can be rectified in some cases by addition of urea. In New Zealand, minimum levels of urea occurs in summer and maximum levels in winter, while in each season, milk from cows in mid-lactation had a lower urea content than milk from cows in early or late lactation.

Relationship Between Milk Lactose and Heat Stability

Conflict in data exists as to the role of lactose in the heat stability of milk. Increasing the lactose content to about 65 g/L reduces heat stability measured by HCT. Removal of lactose by enzyme hydrolysis with

β -galactosidase was found to increase heat stability. This effect is considered to be related to the higher level of reactivity of reducing monosaccharides to participate in Maillard reactions compared to reducing disaccharides such as lactose. Replacement of lactose by sucrose, a nonreducing disaccharide, was also found to reduce heat stability. Seasonal variation of lactose in milk has been reported.

Relationship Between Micelle Size and Heat Stability

The physical organization of the casein proteins of milk into micelles is a major contributor to the heat stability of milk and milk products. It has been suggested that milk with smaller micelles is more heat stable. The smaller, more stable micelles also have a higher proportion of casein as κ -casein.

Heat Stability and Properties of Bulk Milk Products

Milk Powder. The un-denatured whey proteins (WPNI) are used as an indication of the heat treatment given to the milk during powder manufacture. The American Dry Milk Institute specifies powders having ≥ 6 mg un-denatured whey protein nitrogen (N) per gram of powder as low-heat, between 1.51 and 5.99 mg N per gram as minimum heat and ≤ 1.5 mg N per gram as high heat. Several variations in the content of whey proteins and differing denaturation kinetics of different whey proteins at different temperatures can affect WPNI. In general, the WPNI specification is used as an indication of functional properties of milk powders.

UHT Milk. *Effect of season and or stage of lactation:* Age gelation of UHT milk is known to be affected by seasonal/lactational factors, with UHT milk produced in spring or in early lactation tending to gel more quickly. Incidence of burn-on in UHT milk is also associated with late lactation milk. Milks with higher bulk milk cell count (BMCC) gelled before the milk from the same stage of lactation with a lower cell count. Comparison between the most stable milk (late lactation) and both of the least stable milks (early lactation) show that the stable milk is higher in total solids, total protein, casein protein, whey protein, free fatty acids, calcium, and magnesium.

Effect of Protein Component on UHT Milk. A seasonal/lactation pattern with longer gelation times

in the latter part of the season has been reported. Increasing the β -lactoglobulin content by adding a protein fraction rich in β -lactoglobulin or total whey protein content by adding demineralized whey powder caused an increase in the gelation time of some samples. A mechanism suggested to explain this phenomenon includes a greater stabilization on the micelle surface caused by increased β -lactoglobulin denaturation on the micelle surface, or an inhibition of release caused by a higher serum β -lactoglobulin concentration.

Effect of Milk Quality on UHT Milk. Age gelation and the development of bitter flavor defects have also been attributed to the breakdown of milk proteins during storage both by endogenous milk enzymes such as plasmin and exogenous enzymes of bacterial origin which are not denatured by the heating process. Poor milk quality or the presence of bacterial enzymes may lead to premature gelation. Regardless of the effect of bacterial quality on age gelation, its effect on flavor is of great importance and the products of proteolysis have been correlated with the development of bitter flavors.

Effect of Milk Minerals on UHT Milk. The effect of milk minerals on both the heat stability and age gelation is complex. It has been shown that adding calcium chloride increased sedimentation following UHT treatment while addition of orthophosphate, citrate, or EDTA-reduced sedimentation. It was demonstrated that reduced run times (faster fouling) in UHT plants when either calcium or magnesium was added to the milk.

Evaporated Milk. Evaporated milk products are stabilized in their retail container and are perhaps the greatest challenge to the milk processor because of their high solids concentration (typical formulation: 26% solids, 18% MSNF, 8% fat) and the severe heating required to insure commercial sterility (120°C/13 min). The key property of evaporated milk for the consumer is an acceptable viscosity and color without the formation of a gel. However, the greatest concern for manufacturers is the heat stability of milk and its ability to withstand the initial processing. The heat stability of concentrated milk manufacture from either fresh milk or product is subject to a seasonal/lactation variation.

Sweetened Condensed Milk. Sweetened condensed milk is another high solids milk product

although the greatest part of these solids is sucrose. The high ratio of sucrose to water gives the product long shelf life as it inhibits growth of microorganisms and removes the need for a high-heat treatment during manufacture. A key characterization of recombined sweetened condensed milk (RSCM) for both the manufacturer and consumer is its viscosity. The viscosity does not remain constant but tends to increase on storage. An optimal RSCM is one which age thickens to the desirable viscosity in a short time as possible, but remains at the optimum viscosity for as long as possible. The use of homogenization and pasteurization regimes, the use of pH adjustment during powder manufacture and addition of mineral component may have effects on the properties of RSCM.

Yogurt. Yogurt is a gelled milk product by the isoelectric precipitation of milk proteins caused by lowering the pH of the milk to ~ 4.6 . For yogurt manufacture the yogurt milk can be formulated in a number of ways. Once the desired formulation is achieved, the milk is homogenized and heat-treated to destroy undesirable microorganisms and modify the physicochemical properties of milk proteins. Typically, high-heat treatment, such as 85°C for 30 minutes or 95°C for 5 minutes are used. Many of the properties of yogurt are controlled by the heating step. A relatively high-heat treatment is required as a key requirement in that the whey proteins of the milk are denatured. These proteins are then precipitated with the caseins when the pH is lowered by the action of the added cultures. For optimal yogurt performance (highest viscosity, resistance to whey drainage) at least 90% of the whey proteins should be denatured. Although a high-heat treatment is applied, it is essential that there is no precipitation or excessive thickening of the yogurt milk during heating as this will give rise to a product with poor characteristics. In general, increasing the whey protein content of the yogurt milk creates yogurt with a firmer texture and a greater resistance to whey drainage than yogurt made from milk with a normal ratio of casein to whey proteins.

Ice Cream. In ice cream, milk fat components play some essential roles in flavor and structure/texture. These roles are performed by the milk fat through a variety of physical interactions at the surfaces of air bubbles and by clumping of fat globules. Milk proteins are involved in this role through their surface-active properties, stabilizing the air/water interface of air bubbles and the oil/water interphase of fat glob-

ules. The balance of surface-active material in the ice cream mix is further manipulated by low molecular weight emulsifier added to the formulation.

QUALITY AND SAFETY TESTS AND FUTURE TRENDS

Liquid Milk

Hazard analysis critical control points (HACCP) is a valuable tool for assuring milk quality and safety. HACCP comprises three key areas: hazard identification, determination of critical control points (CCP), and monitoring CCP. An example of a HACCP audit chart for raw milk is given in Table 4.12.

The critical operation in raw milk is the receipt of milk at the receipt bay of the dairy processing plant. The potential risks include mainly contamination of milk with antibiotics, spoilage microorganisms, poor organoleptic quality and adulteration of milk, and separation of fat during transport of raw milk. The purpose of CCP is essentially to assure quality and stability of milk for processing. The prevention control and monitoring include a variety of visual inspection, chemical and physical tests, microbiological and organoleptic tests. Possible corrective actions are also shown in Table 4.12. For example, when presence of antibiotics is detected in the milk, the quality assurance laboratory should inform the factory manager and withhold the product from sale.

An example of HACCP audit chart for pasteurization and homogenization of raw milk is given in Table 4.13. The critical control points in assuring quality and safety are pasteurization, homogenization, cooling, filling and storage of packaged milk. The potential risks involved include survival of pathogen due to improper pasteurization time/temperature control, separation of cream due to improper homogenization, bacterial growth in milk during cooling and temporary storage before filling operations, microbial contamination of filled milk due to inadequate plant hygiene and sanitation, contamination of end product with sanitizers, under and overfilling, use-by-date labeling errors or defects, packaging defects, and microbial growth during storage of packaged milk before retail distribution to supermarkets. The prevention control and monitoring include visual inspection, chemical, physical, and microbiological tests and checking equipment including heat exchangers, fillers and packaging machinery.

In the processing of flavored milk, similar quality assurance tests are conducted. An additional critical

Table 4.12. HACCP Audit Chart for Raw Milk (Critical Operation: Receive)

Potential Risk	Critical Control Point	Prevention, Control, and Monitoring	Corrective Action
Presence of antibiotics Unsatisfactory microbial quality	Farm milk quality	Delvo test ($<0.002 \mu\text{g/mL}$)	Inform, withhold product
	Farm milk quality	Direct microscopic count ($<500,000$ clumps/mL)	Inform, withhold product
Poor organoleptic quality Nonstandard chemical composition	Farm milk quality	Check aroma, color (fresh and clean)	Reject milk for further processing
	Farm milk quality	Fat $> 3.2\%$; protein $> 3.1\%$; Freezing point less than -0.517°C	Inform, standardize milk
Growth of spoilage microbes	Plant sanitation	Visual inspection of vat, biotrace swabbing	Re-wash, sanitize, check refrigeration
	Refrigeration control	Visual check (odor, color)	Mechanical problem
Separation of fat and lipolysis High microbial count	Agitation	SPC $< 150,000 \text{ CFU/mL}$ Coliforms	Inform, contact farm milk supplier
	Farm milk quality	$< 100 \text{ CFU/mL}$	
High thermophilic; Psychrotrophic count	Farm milk quality	Thermophilic $< 20,000 \text{ CFU/mL}$;	Inform, contact farm milk supplier
		Psychrotrophic $< 10,000 \text{ CFU/mL}$	

Table 4.13. HACCP Audit Chart: Pasteurization/Homogenization of Raw Milk

Critical Operation	Potential Risks	Critical Control Point	Prevention, Control, and Monitoring	Corrective Action
Pasteurization	Pathogen survival	Time/Temperature control	Pasteurization temperature not < 72.5°C; phosphatase activity < 10 µg/mL p-nitrophenol	Re-sterilize pasteurizer, check boiler, diversion valve, re-pasteurize milk
Homogenization	Separation of cream	Homogenizer pressure	Check gauge readings, 1st stage: 500 psi; 2nd stage: 2,000 psi Homogenization index < 10	Adjust homogenizer pressure
Cooling	Bacterial growth	Low temperature	Milk temperature < 3°C	Use back up compressor
Storage and fillin	Microbial contamination	Cooling Plant hygiene Plant sanitation	Vat temperature < 4°C Effective cleaning, visual check Vat sanitizing, check operation of spray ball Correct concentration of sanitizers SPC < 50,000 CFU/mL; Coliforms < 1/mL	Check temperature and cool immediately Re-clean vat Use manual cleaning with sanitizers Check dosing and mixing procedure Inform factory manager, check CIP
	Sanitizer contamination	Flush residual sanitizer	Visual check CIP Fat < 3.2%; freezing point less than -0.517°C, free of odor and taints Check volume/weight within ± 10 g. Check carton machine rubbers 14 days from packing date	Staff training Withhold product
	Under/over fillin cartons	Product weight		Adjust fill settings, replace carton machine rubbers
	Incorrect/illegible use by date	Coder error		Re-set coder
	Packaging defect	Sealing of cartons	Check packaging, squeeze test	Withhold affected cartons, inform
Storage of packed milk	Microbial growth	Temperature control	Cool room temperature < 2°C, insure doors closed properly	Inform, mechanical fault, check refrigeration

operation in the manufacture of fl vored milks is the addition of fl vors to pasteurized milks. This may lead to postpasteurization microbial contamination and if fl vor is contaminated, the end product will have uncharacteristic fl vors and possibly microbial growth.

DAIRY PRODUCTS

In cheese manufacture, acidity/pH is important process controls for the development of quality cheese. The development of acidity is measured by titratable acidity using phenolphthalein and expressed as percent lactic acid. The various manufacturing steps in the cheese production are determined by acid development. In some cases a casein–fat ratio is determined before the cheese manufacture and milk is standardized to standard casein: fat ratio to obtain specific cheese varieties. The end product is tested for fat, moisture, pH, and salt content. In some countries such as Australia, certain standards are set for fat-in-dry-matter of cheese. For example, all export Cheddar cheese should contain a minimum of 50% fat-in-dry-matter.

In yogurt manufacture, the pH is used as a process control to monitor the fermentation process. In some countries, the final pH of yogurt should be <4.5 . This is used as a safety measure to prevent growth of pathogens. Composition of the milk and the ingredients, such as skim milk powder is required to prepare different yogurt mixes which varies according to total solids basis or total nonfat-solids basis. The fat content of milk is essential to standardize the yogurt mix to a set level of fat content, particularly in the manufacture of reduced-fat or low-fat yogurts.

In the manufacture of ice cream, the composition of the butter fat, milk SNF of the milk, and cream and skim milk powder are determined. One of the key process controls in ice cream manufacture is the overrun. The ice cream is cooled and aged, after which it is pumped through an ice cream continuous freezer to obtain desired overrun and then the freezer is set at this overrun for the rest of the fillin operations.

Microbiological tests in dairy quality labs involve SPC and coliform tests. The results should conform within stipulated standards. This may vary in different countries. SPC determines the level of total bacteria in the product; however, coliform counts correlate with the hygiene and sanitation practices of the product manufacture. The organism commonly accepted as belonging to the coliform group predominantly occurs only in the intestines of homeothermic ani-

mals. The presence, therefore, of these organisms in raw milk is a strong indication of fecal contamination and generally unhygienic conditions. Their presence is a sign of poor sanitation and ineffective control of plant hygiene.

In both SPC and coliform tests, a quantity of prepared samples is placed aseptically into a petri dish containing nutrients or selective agar. The bacteria are dispersed throughout the agar and after a period of incubation, the plate is examined for colonies, which are counted and multiplied by the dilution factor to estimate the original bacterial concentration. The psychrotrophic organisms (cold surviving) have become increasingly important over the past years with the increase in bulk storage under refrigerated conditions. This is now widely adopted in testing of milk and dairy product manufacture as a quality and safety test. In some product manufacture, thermophilic bacterial counts are tested. In some cases such as yogurt and cheese, yeast and mould counts are also determined as a quality and safety test.

Direct microscopic count is useful for routine examination of milk. This test is useful for its ease and speed and its ability to detect anomalies in the milk such as leucocytes in mastitis, and so forth. However, it is not a viable count and dead organisms are also counted, which results in unreliable estimates of some products, such as pasteurized milk.

FUTURE TRENDS

Milk is increasingly considered as a basket of pharma- and nutraceuticals as more clinical evidence emerges on the therapeutic benefit of milk and milk-derived ingredients. As consumers are becoming more aware of health and benefit from dairy foods in the prevention of contemporary diseases, the global market for dairy foods and nutraceuticals will increasingly grow worldwide. Hence, the compositional analysis, and development of new ingredients and formulations will become increasingly important. New technique will need to be developed to extract the bioactive and therapeutic components in milk, process them without affecting their bioactivity, protect them and deliver them in active and/or viable forms to the consumer. As new bioactive and therapeutic dairy-derived ingredients are developed and tested for efficacy, new and improved analytical techniques will need to be developed. The quality and safety issues of any new dairy-based ingredient will need to be closely monitored. Bioterrorism is another area of concern for the dairy processing

industry. The dairy industry not only needs to be vigilant but also develop new and rapid online testing to identify chemical or biological contaminants.

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5

Microbiological Considerations Related to Dairy Processing

Ronald H. Schmidt

Introduction
Raw Milk Microflora
General Characteristics of Raw Milk Microorganisms
Pathogens Associated With Milk and Milk Products
General Characteristics
Impact of Foodborne Illness on Dairy Industry Practices and Regulations
Impact of Microorganisms on The Quality and Spoilage of Milk and Milk Products
General Enzymatic Reactions
Impact of Specific Classes of Microorganisms on Quality and Spoilage
The Gram-Negative Rods
Microbiological Analyses of Milk and Milk Products
Processing and Handling Interventions
Current and Future Microbiological Issues
References

INTRODUCTION

Composition, nutritional value, and other intrinsic factors, make milk and many milk products attractive for the growth of a variety of microorganisms. Because of microbiological food safety implications, the dairy industry is a highly regulated food industry in the United States, as well as throughout the developed world. In the United States, strict pasteurization and sanitation regulations are based upon the Grade A Pasteurized Milk Ordinance (PMO, 2005), which is adopted into state and local regulations, and is the regulatory standard under the National Conference on Interstate Milk Shipments (NCIMS, 2007), a cooperative state/federal program. This regulatory program covers primarily Grade A fluid milk and fluid milk products which are defined under the PMO. State regulatory programs are in effect for ice cream, and manufactured dairy products not cov-

ered by the PMO. The U.S. Department of Agriculture (USDA)/Agricultural Marketing Service (AMS) offers a voluntary inspection and grading program for manufactured dairy products (e.g., cheese, butter, cheese, nonfat dry milk), and provides recommended criteria and standards for raw milk for manufacturing purposes (e.g., non-Grade A milk), for adoption by state regulatory agencies (USDA, 2007).

Many innovations in pasteurization, sanitation practices, sanitary design, refrigeration, and process design have evolved and continue to evolve in the dairy industry to minimize microbiological contamination and to assure safe, high-quality dairy products. This chapter will provide an overview of dairy product microbiology, as well as sanitation, testing, and processing considerations related to these microorganisms.

A diverse array of microorganisms has been isolated from, or has been associated with, milk and milk products (Cousin, 1982; Gilmour and Rowe, 1990; Griffiths 2000; Jay, 2000). A partial listing of these microorganisms and their general characteristics, source, and potential significance are presented in Tables 5.1–5.4.

RAW MILK MICROFLORA

The type and level of microorganisms present in raw milk (e.g., raw milk microflora) is highly varied and depends upon the following on-farm factors:

- Cow health (e.g., mastitis, other)
- Cow diet and feeding practices
- Milking procedures and practices
- Milk contact surfaces of equipment
- Sanitation practices

Table 5.1. Gram-Negative, Nonspore-Forming Microorganisms^a Associated with Milk and Some of Their General Characteristics

Microorganism	General Characteristics	Source	Significance
<i>Section 1—Spirochaeta</i>			
Genus <i>Leptospira</i> <i>Leptospira interrogans</i>	Flexible helicoidal rods; obligate aerobe ^b ; growth temperature: optimum 28–30°C	Animal-borne; infected cattle	Animal pathogen causing <i>Leptospirosis</i> in cattle; Human pathogen; association with pasteurized milk products is rare
<i>Section 2—Aerobic/Microaerophilic, Motile, Helical/Vibroid, Gram-Negative Bacteria</i>			
Genus <i>Campylobacter</i> <i>C. coli</i>	Spiral curved rods; microaerophilic ^c ; growth temperature: optimum 37–42°C	Animal intestinal tract and feces	Human pathogen associated with foodborne illness, especially from raw milk consumption; association with pasteurized milk products is rare
<i>Section 4—Gram-Negative Aerobic Rods and Cocci</i>			
Genus <i>Acinetobacter</i>	Short plump rods; aerobic; growth temperature: optimum 25–35°C psychrotroph ^d (certain strains); some proteolysis	Soil, water, environment; milking equipment	Minor cause of spoilage of milk and milk products; associated with ropy defect in milk
Genus <i>Alcaligenes</i>	Rods or cocci; obligate aerobe; growth temperature: optimum 20–37°C, psychrotroph	Soil, water, environment, milking equipment	Associated with rotten eggs; role in milk and milk product spoilage not clearly established
Genus <i>Aeromonas</i> <i>Aeromonas putrefaciens</i> (Formerly named <i>Pseudomonas putrefaciens</i>)	Rods; aerobic; growth temperature: optimum 20–25°C, psychrotroph; no growth at low salt concentrations; proteolytic	Soil, water, and environment	Spoilage of milk products; cause of surface taint in butter
Genus <i>Brucella</i> <i>Brucella abortus</i> , <i>Bru. melitensis</i>	Rods; aerobic; growth temperature: optimum 37°C	Infected animals, animal wastes	Animal pathogen— <i>Brucellosis</i> in lactating animals; human pathogen— <i>Undulant Fever</i> ; association with pasteurized milk products is rare

Genus <i>Flavobacterium</i>	Rods; aerobic; growth temperature: optimum 20–30°C, psychrotroph; proteolytic	Soil, water, and environment	Spoilage of refrigerated milk and milk products
Genus <i>Moraxella</i>	Short plump rods; aerobic; growth temperature: optimum 25–35°C, psychrotroph	Soil, water, environment	Role in spoilage of milk and milk products not clearly established
Genus <i>Pseudomonas</i> Psychrotrophic <i>Pseudomonas fluo escens</i> , <i>Ps. fragi</i> , <i>Ps. putida</i>	Straight or curved rods; obligate aerobic; growth temperatures: optimum 25–30°C; proteolytic and lipolytic; heat-stable extracellular enzymes (proteinases, lipases)	Soil, water, environment; milking equipment	Spoilage of milk and milk products; proteolytic spoilage; bitter fl vor and gelation of ultrahigh temperature (UHT) products; slimy defect in cottage cheese. Fruity aromas and off-fl vors
Nonpsychrotrophic <i>Ps. aeruginosa</i>	Growth temperatures: optimum 35–37°C	Soil, water, environment	Causes mastitis in cattle; human pathogen—usually water-borne
Section 5—Facultative Anaerobic Gram-Negative Rods			
Genus <i>Aeromonas</i> <i>Aeromonas hydrophila</i>	Rods; facultative anerobe ^e ; growth temperature (high variation between strains): optimum 20–35°C, psychrotroph	Water and environment	Human pathogen (usually through the skin) causing wound infections. Association with illness from water consumption not clearly established; association with pasteurized milk products is rare
Genus <i>Chromobacterium</i> <i>Chromobacterium violaceum</i>	Rods; facultative anaerobe; violet pigmentation (some nonpigmented strains; growth temperature: optimum 30–35°C	Soil, water, environment	Human pathogen; usually soil or water-borne; association with pasteurized milk products is rare
Genus <i>Citrobacter</i> <i>Citrobacter amalonacticus</i> , <i>Ci. diversus</i> , <i>Ci. freundii</i>	Gram-negative rods; facultative anaerobe; growth temperature: optimum 28–30°C	Water, sewage, animal wastes	Human pathogen associated with nosocomial infections ^f ; not a significant cause of foodborne illness

(cont.)

Table 5.1. (cont.)

Microorganism	General Characteristics	Source	Significance
Genus <i>Enterobacter</i> <i>Enterobacter aerogenes</i> , <i>En. agglomerans</i> , <i>En. amnigenus</i> <i>En. cloacae</i> , <i>En. intermedium</i> , <i>En. sakazakii</i>	Gram-negative rods; facultative anaerobes; growth temperature: optimum 37–44°C	Water, sewage, animal wastes; mastitis; milking equipment	<i>E. sakazakii</i> —Human pathogen (usually nosocomial) in hospitalized infants, associated with contaminated dried infant formulae
Genus <i>Escherichia</i> <i>Escherichia coli</i>	Gram-negative rods; facultative anaerobe; growth temperature: optimum 37°C (some strains have lower optimum growth); numerous strains with varied characteristics	Animal intestinal tract; animal wastes; bedding; milking equipment	Several types are human pathogens and associated with foodborne illness outbreaks; causes spoilage with gas production in milk products, especially cheese
Genus <i>Salmonella</i> <i>Salmonella bongori</i> , <i>S. enterica</i>	Gram-negative rods; facultative anaerobes; greater than 2,400 serovars; growth temperature: optimum 37°C; survival at low water activity	Animal intestinal tract; animal feces	Human pathogen— <i>S. enterica</i> subspecies and serovars e.g., <i>S. enterica</i> serotype enteritidis (<i>S. enteritidis</i>) and <i>S. enterica</i> serotype typhimurium (<i>S. typhimurium</i>); associated with foodborne illness outbreaks
Genus <i>Yersinia enterocolitica</i> <i>Yersinia enterocolitica</i> Group— <i>Yersinia enterocolitica</i> , <i>Y. intermedia</i> , <i>Y. fredericksonii</i> , <i>Y. kristensenii</i>	Gram-negative rods; facultative anaerobes; growth temperature: optimum 28–29°C; psychrotroph	Water, sewage, animal feces (especially hogs)	Human pathogen associated with foodborne illness, especially from raw milk consumption; association with pasteurized milk and milk products is rare

^aClassified according to *Bergey's Manual of Determinative Bacteriology* (Bergey et al., 1994).

^bBacteria that require oxygen levels of 20% or higher for growth.

^cBacteria that need oxygen for growth, but grow best at, or may even require, reduced oxygen tensions (e.g., 10% or less).

^dDefine in a variety of ways. The generally accepted definition of *psychrotroph* in dairy systems is a bacterium with optimum growth at higher temperatures, but will grow at temperatures at 7°C or less (differentiated from a *psychrophile* that has its temperature optimum at cold temperatures).

^eBacteria that can grow with or without free oxygen (differentiated from obligate anaerobes that are poisoned by oxygen).

^fGenerally define as infections in hospitalized patients, from contamination sources in the hospital.

Table 5.2. Gram-Positive, Nonspore-Forming Microorganisms Associated with Milk and Their General Characteristics

Microorganism	General Characteristics	Source	Significance
<i>Section 10—Mycoplasma</i>			
Genus <i>Mycoplasma</i>			
<i>Mycoplasma bovis</i>	Pleomorphic cocci; usually acellular (lacking cell wall); growth temperature: optimum 35–37°C	Infected animals, animal wastes and environment	Causes mastitis in cattle; human pathogen; association with pasteurized milk products is rare
<i>Section 12—Gram-Positive Cocci</i>			
Genus <i>Enterococcus</i> ^a			
<i>Enterococcus faecalis</i> (Formerly <i>Streptococcus faecalis</i>)	Cocci chains; facultative anaerobe; growth temperature: optimum 35–37°C	Animal wastes; water and environment	Causes mastitis in cattle; human pathogen often water-borne (lake and beach water); association with pasteurized milk products is rare
Genus <i>Lactococcus</i> ^b			
<i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>Lac. lactis</i> subsp. <i>cremoris</i>	Gram-positive cocci; facultative anaerobe	Feedstuffs, silage, farm environment	Mesophilic starter culture used in cheese and fermented dairy products
<i>Lac. lactis</i> biovar <i>diacetylactis</i>	Growth temperature: optimum 25–30°C; lactic acid bacteria (LAB), homofermentative ^c		Mesophilic aroma culture in cultured dairy products. Used in cottage cheese to inhibit <i>Pseudomonas</i> slime formation
Genus <i>Leuconostoc</i>			
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> , <i>Leu. mesenteroides</i> subsp. <i>dextranicum</i>	Growth temperature: optimum 18–25°C; LAB, heterofermentative ^d ; produce diacetyl and from citrate	Feedstuffs, silage, and farm environment	Produce extracellular dextran from sucrose on fruits and vegetables (to a lesser extent in dairy isolates)

(cont.)

Table 5.2. (cont.)

Microorganism	General Characteristics	Source	Significance
<i>Leu. mesenteroides</i> subsp. <i>cremoris</i>	Growth temperature: optimum 20–30°C; LAB, heterofermentative	Fruits and vegetables raw milk	Mesophilic aroma culture in cultured dairy products. Used in cottage cheese to inhibit <i>Pseudomonas</i> slime formation; no dextran production from sucrose
<i>Leu. paramesenteroides</i> ; <i>Leu. lactis</i>	Gram-positive cocci; facultative anaerobe; growth temperature: optimum 20–30°C; LAB, heterofermentative	Fruits and vegetables raw milk	Not used as dairy starter cultures
<i>Leu. citrovorum</i>	Gram-positive cocci; facultative anaerobe; growth temperature: optimum 20–30°C; LAB, heterofermentative, citrate fermentation to diacetyl	Fruits and vegetables raw milk	Used as a buttermilk starter culture
Genus <i>Micrococcus</i>			
<i>Micrococcus varians</i>	Cocci bunched or clusters; obligate aerobe; growth temperature: optimum 25–37°C	Animal-borne; human skin	Not generally pathogenic
Genus <i>Staphylococcus</i>			
<i>Staphylococcus aureus</i>	Cocci bunched or clusters (grape-like); aerobe or facultative anaerobe; growth temperature: optimum 30–37°C Growth stimulated by salt levels; produces heat stable enterotoxin	Animal-borne; human skin	Associated with mastitis in cattle; human pathogen—associated with foodborne intoxications from products which have human contact and temperature abuse for toxin production
<i>Staph. caprae</i> , <i>Staph. chromogenes</i> , <i>Staph. epidermidis</i> , <i>Staph. hyicus</i>	Growth temperature: optimum 30–37°C	<i>Staph. caprae</i> —goats	While some are potentially pathogenic, these species have less association with foodborne illness than <i>S. aureus</i> .

Genus <i>Streptococcus</i>	Cocci chains; facultative anaerobe		
Mesophilic/Hemolytic—human pathogens, <i>Str. pyogenes</i>	Growth temperature: optimum 35–37°C	Animals; humans.	Causes scarlet fever, sometimes associated with foodborne transmission
<i>Str. suis</i> , <i>Str. zooepidermicus</i>			Sometimes associated with foodborne illness outbreaks (raw milk cheese)
Mesophilic/Hemolytic—animal pathogens, <i>Str. agalactiae</i> , <i>Str. dysgalactiae</i> , <i>Str. uberis</i>		Animals; humans	Associated with mastitis in cattle and humans; rarely associated with foodborne illness
Thermophilic/Nonhemolytic <i>Str. thermophilus</i>	Cocci chains; facultative anaerobe; growth temperature: optimum 37–50°C; LAB, homofermentative	Saliva, farm environment	Thermophilic starter culture used in yogurt, and Swiss-type and Italian cheese
Section 14—Gram-Positive Regular Formed, Nonsporulated Rods			
Genus <i>Lactobacillus</i>	Gram-positive rods; anaerobe/microaerophilic	Feedstuffs, silage, and farm environment	
<i>Lactobacillus delbruekii</i> subsp. <i>lactis</i> , <i>Lb. helveticus</i>	Growth temperature: optimum 40–43°C; LAB, usually homofermentative (some strains heterofermentative)		Thermophilic starter culture used in Swiss-type and Italian cheese or nonstarter LAB (NSLAB) in cheese
<i>Lb. delbruekii</i> subsp. <i>bulgaricus</i>	Growth temperature: optimum 40–43°C; LAB, usually homofermentative (rarely heterofermentative)		Thermophilic starter culture used in yogurt, and Swiss-type and Italian cheese
<i>Lb. acidophilus</i>	Growth temperature: optimum 45°C; LAB, usually homofermentative (rarely heterofermentative)		Thermophilic starter culture used in acidophilus milk and related probiotic dairy products; kefi, yogurt
<i>Lb. casei</i>	Growth temperature: optimum 30–37°C; LAB, usually homofermentative, and some strains are heterofermentative and produce diacetyl		Mesophilic starter used in a variety of dairy products; probiotic strains
<i>Lb. curvatus</i>	Growth temperature: optimum 30–35°C; LAB, homofermentative		NSLAB in cheese; associated with calcium lactate crystals on cheddar cheese
<i>Lb. plantarum</i>	Growth temperature: optimum 30–35°C; LAB, homofermentative (some strains heterofermentative)		Used in fermented vegetable products, NSLAB in cheese

(cont.)

Table 5.2. (cont.)

Microorganism	General Characteristics	Source	Significance
Kefi -associated lactobacilli ^e	Generally heterofermentative (some variation), certain species not active lactose fermenters (e.g., <i>Lb. breves</i>)		Isolated from Kefi and related products. May be associated in NSLAB in cheese
<i>Lb. fermentum</i>	Growth temperature: optimum 41–42°C; LAB, not an active lactose fermenter		Associated with blow-hole defect in certain cheeses; also associated with Kefi manufacture
Genus <i>Listeria</i>			
<i>Listeria monocytogenes</i> , <i>L. innocua</i> , and others	Short rods; facultative anaerobe; growth temperature: optimum 37°C, psychrotroph; salt tolerant	Soil, water, and environment	<i>L. monocytogenes</i> causes <i>circling disease</i> in cattle and <i>Listeriosis</i> (especially in immuno-compromised, elderly and neonates)
Section 15—Gram-Positive Irregular Formed, Nonsporulated Rods			
Genus <i>Actinomyces</i>	Rods; aerobic	Animal-borne	
<i>Actinomyces bovis</i>	Growth temperature: optimum 36°C		Causes “lump jaw” in cattle. Not associated with human disease
<i>Act. pyogenes</i> (formerly <i>Corynebacterium pyogenes</i>)	Growth temperature: optimum 30°C; proteolytic		Implicated in “summer mastitis” in cattle; human pathogen; minor role in spoilage reactions
Genus <i>Athrobacter</i> (sometimes termed “Coryneform”)	Rod-cocci; growth temperature: optimum 25–30°C, psychrotroph	Soil, water and environment	Certain species are human pathogens; not highly significant in milk and milk products
Genus <i>Aureobacterium</i>			
<i>Aureobacterium liquefaciens</i>	Short slender rods; obligate aerobic; growth temperature: optimum 25–30°C	Farm environment	Spoilage—produces bright yellow pigment
Genus <i>Bifidobacteriu</i> Greater than 30 species. Certain species are associated with milk and milk products ^f	Pleomorphic rods; anaerobe/facultative anaerobe; growth temperature: optimum 37–41°C; LAB, heterofermentative	Colon of animals and humans	Probiotic dairy products

Genus <i>Brevibacterium</i>				
<i>Brevibacterium linens</i>	Rods-cocci; obligate aerobe; growth temperature: optimum 20–30°C; yellow to deep orange-red pigment (incorrectly referred to as “red mold”)	Animals; human skin	Proteolysis and flavor development in surface-ripened cheese (Limburger type)	
Genus <i>Caseobacter</i>	Irregular rods; obligate aerobe; growth temperature: optimum 25–30°C	Environmental	May contribute to ripening of soft cheeses varieties; associated with the rind of soft cheeses	
Genus <i>Corynebacterium</i>				
<i>Corynebacterium bovis</i> , <i>Corynebacterium striatum</i>	Straight and curved rods; facultative anaerobe; growth temperature: optimum 37°C	Animal-borne	Cause of mastitis in cattle Minor significance in milk and milk products	
Genus <i>Microbacterium</i>				
<i>Microbacterium lacticum</i>	Irregular rods; generally aerobe (may be weakly anaerobe); growth temperature: optimum 30°C	Soil and environment; animal-borne	Associated with mastitis in cattle Minor significance in milk and milk products	
Genus <i>Propionibacterium</i>				
<i>Propionibacterium freudenreichii</i> subsp. <i>shermanii</i>	Pleomorphic rods; anaerobe to aerotolerant; growth temperature: optimum 30°C; metabolizes lactate to form propionate and carbon dioxide	Soil, silage, farm environment	Flavor and eye formation in Swiss-type cheese	
Section 16—The <i>Mycobacteria</i>				
Genus <i>Mycobacterium</i>	Slightly curved or straight rods; aerobe; growth temperature: optimum 37°C	Animal and human intestinal tract; cattle manure	Human pathogen—tuberculosis; generally nonpathogenic to animals	
<i>Mycobacterium tuberculosis</i>			Causes tuberculosis in man, goats, and cattle	
<i>Mycobacterium bovis</i>			Causes <i>Johne's disease</i> in cattle; significance as a human pathogen (<i>Crohn's disease</i>) is unknown, and is under investigation	
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP)				

^aFormerly Group D *Streptococcus*. ^bFormerly Group N *Streptococcus*. ^cFerments glucose to lactic acid as primary metabolite. ^dFerments glucose to lactic acid, acetic acid, and carbon dioxide. ^eLactobacilli commonly associated with Kefir manufacture include *Lb. brevis*, *Lb. cellobiosus*, *Lb. higaridii*, *Lb. kefir*, *Lb. kefi anfaciens*, *Lb. kefi granum*, *Lb. parcasei*, and *Lb. parakefi*. ^fMost common species in dairy systems include *Bifidobacterium adolescentis*, *Bf. animalis*, *Bf. bifidus*, *Bf. breve*, *Bf. infantis*, *Bf. lactis*, *Bf. Longum*, and *Bf. thermophilum*.

Table 5.3. Spore-Forming Microorganisms Associated with Milk and Some of Their General Characteristics

Microorganism	General Characteristics	Source	Significance
<i>Section 9—The Rickettsias and Chlamydias</i>			
Genus <i>Coxiella</i>	Pleomorphic rods; Gram-negative; exists in sporulated state in soil and environment; obligate intracellular; parasitic; acidophile; excellent survival even after prolonged exposure to environmental extremes (e.g., elevated temperature, desiccation, osmotic shock, ultraviolet light, and chemical sanitizers)	Infected animals; soil and environment	Human pathogen causing Q-fever (Query fever), most commonly by aerosol route, or, less commonly, by passing through milk; heat inactivation target organism for pasteurization temperature and time requirements in the United States
<i>Section 13—Endospore Producing Gram-Positive Rods and Cocci</i>			
Genus <i>Bacillus</i>	Gram-positive rods; aerobic	Soil, feed, silage, and environment	Human pathogen associated with foodborne illness Spoilage of milk and milk products; associated with “bitty” cream defect in cream, and “sweet curdling” defect in milk
<i>Bacillus cereus</i>	Growth temperature: optimum 20–35°C; heat-resistant endospore; proteolytic		Spoilage of milk and milk products; associated with ropiness or sliminess, and other spoilage reactions in raw, pasteurized, ultra-pasteurized, and ultrahigh temperature (UHT) processed milk products
<i>B. circulans</i> , <i>B. coagulans</i> , <i>B. firmus</i> , <i>B. licheniformis</i> , <i>B. subtilis</i>	Growth temperature: optimum 20–35°C, some stains psychrotrophic; proteolytic; <i>B. coagulans</i> ferments glucose to lactic acid		Spoilage of canned foods (“flat-sour spoilage”); rarely associated with milk products
<i>B. stearothermophilus</i>	Growth temperature: optimum 46–65°C (thermophile); heat-resistant endospore; proteolytic		
Genus <i>Clostridium</i>	Gram-positive rods; strict anaerobe	Soil, feed, silage, and environment	Spoilage of milk and milk products, especially canned products; associated with “late blowing” due to extensive gas formation and rancidity in ripened cheese
<i>Clostridium butyricum</i> , <i>Cl. tyrobutyricum</i> , <i>Cl. sporogenes</i>	Growth temperature: optimum 30–40°C; carbohydrate fermentation (acetic acid), convert lactic acid to butyric acid; some strains lipolytic		Human pathogen associated with foodborne illness
<i>Cl. perfringens</i>	Growth temperature: optimum 30–40°C		

Table 5.4. Fungi and Algae Associated with Milk and Their General Characteristics and Significance

Microorganism	General Characteristics and Significance
<i>Yeasts</i>	
Genus <i>Candida</i>	
<i>Candida albicans</i>	Associated with sporadic mastitis in cattle. Minor significance in milk and milk products
<i>Candida kefyr</i> , <i>Candida friedricchi</i> , <i>Candida holmii</i> , <i>Candida krusei</i> , <i>Candida pseudotropicalis</i> , <i>Candida rancens</i> , <i>Candida tenuis</i>	Associated with manufacture of Kefi and related products
<i>Candida lacticondensi</i> (formerly <i>Toruposis lactis-condensi</i>)	Associated with spoilage of sweetened condensed milk
<i>Candida famata</i> , <i>Candida valida</i>	Associated with the ripening of certain blue veined cheeses
Genus <i>Debaryomyces</i>	
<i>Debaryomyces hansenii</i>	Contributes to ripening and flavor of certain cheeses; cause of gassiness in cheese and related products
Genus <i>Hansenula</i>	
<i>Hansenula mrakii</i> (<i>Williopsis mrakii</i>)	Associated with Kefi manufacture and cheese ripening
Genus <i>Kluyveromyces</i>	
<i>Kluyveromyces bulgaricus</i> ,	Associated with the manufacture of Kefi and related products
<i>K. marxianus</i> var. <i>marxianus</i> (formerly <i>K. fragilis</i>)	Associated with the manufacture of Kefi and related products; cause of gassy spoilage of yogurt
<i>K. marxianus</i> var. <i>lactis</i> (formerly <i>K. lactis</i>)	Associated with ripening of some cheese; cause of gassy spoilage in yogurt, buttermilk, cheese
Genus <i>Pichia</i>	
<i>Pichia farinose</i>	Gassy spoilage in yogurt and cultured dairy products
Genus <i>Rhodotorula</i>	
<i>Rhodotorula rubrum</i> , <i>Rh. glutinis</i> , <i>Rh. mucilaginosa</i>	Gassy spoilage of yogurt and cultured dairy products
Genus <i>Saccharomyces</i>	
<i>Saccharomyces carlsbergensis</i> , <i>Sacc. cerevisiae</i> , <i>Sacc. bayanus</i>	Brewers and bakers yeast; associated with the manufacture of Kefi and related products
<i>Sacc. flo entius</i> , <i>Sacc. lactis</i> , <i>Sacc. unisporus</i>	Associated with the manufacture of Kefi and related products
Genus <i>Sporobolomyces</i>	
<i>Sporobolomyces roseus</i>	Gassy spoilage of cultured dairy products

Table 5.4. (cont.)

Microorganism	General Characteristics and Significance
Genus <i>Torulopsis</i>	
<i>Torulopsis holmii</i> (sometimes referred to as <i>Sacc. subsp. torulopsis holmii</i>)	Associated with Kefi manufacture; Gassy spoilage of cultured dairy products
<i>Torulopsis etchellsii</i>	Used with <i>Penicillium roquefortii</i> in production of blue cheese flavoring preparations
Molds	
Genus <i>Aspergillus</i>	
<i>Aspergillus flavus</i>	Associated with feed, especially corn; produces aflatoxin (potential carcinogens); aflatoxin testing of raw milk is required by regulation in the United States and other parts of the world
Genus <i>Cladosporium</i>	
<i>Cladosporium fulvum</i> , <i>Cladosporium herbarium</i>	Cheese ripening Associated with spoilage of cultured dairy products
Genus <i>Geotrichum</i>	
<i>Geotrichum candidum</i> (e.g., “Machinery Mold”)	White, yeast-like colonies (often classed as a yeast), sometimes turn black; associated with ripening of surface ripened cheeses
Genus <i>Penicillium</i>	
<i>Penicillium roqueforti</i> , <i>Pen. glaucum</i>	Blue-green mold; ripening of blue-veined cheese varieties
<i>Pen. casei</i>	Yellow-brown mold; associated with some Swiss cheeses
<i>Pen. Album</i> , <i>Pen. camemberti</i> , <i>Pen. candida</i> (<i>candidum</i>), <i>Pen. caseicola</i> (<i>caseicolum</i>)	Grayish white or snow white (e.g., <i>Pen. candidum</i>) mold; ripening of surface ripened soft cheese varieties
Genus <i>Rhizopus</i>	
<i>Rhizopus stolonifer</i>	White turning to black; associated with stale bread; general spoilage of dairy products
Genus <i>Scopulariopsis</i>	
<i>Scopulariopsis brevicaulis</i>	Grayish white turning to yellowish-brown; highly proteolytic; causes ammonia off-flavor in mold-ripened cheese
Genus <i>Sporendonema</i>	
<i>Sporendonema sebi</i>	Discrete “button” colonies; associated with spoilage of sweetened condensed milk
Algae	
Genus <i>Prototheca</i>	
<i>Prototheca zopfii</i> , <i>P. wickerhamii</i>	Achlorophytic unicellular algae; associated with sporadic mastitis in cattle. Human pathogen (immunocompromised host) especially through skin and wound infections

- Refrigeration practices
- Holding time prior to processing
- Season of the year
- Climate and geography

The majority of microorganisms isolated from raw milk are bacteria, although other microorganisms (e.g., viruses, protozoan parasites, algae, yeasts, molds) have also been associated with raw milk. In early years, the “natural microflora” and its impact on the ripening quality of cheese and fermented products was investigated (Dahlberg and Marquardt, 1942). At that time, lactic acid bacteria (LAB) from farm sources were primary components of the raw milk microflora. However, with improved sanitation and refrigeration practices, and adaptation and selection for other microorganisms, the raw milk microflora is quite different today compared to early times. Most notably, the levels of LAB in today’s raw milk are usually at much lower numbers than was historically seen. These bacteria are susceptible to today’s on-farm sanitation practices, and do not grow well under refrigeration, and thus give way to psychrotrophic microorganisms that grow at low temperatures.

The raw milk microflora may either directly or indirectly affect the quality and safety of pasteurized milk and milk products as follows:

- *Direct impact:* Presence of a sufficient level of heat-resistant spoilage and pathogenic microorganisms that survive pasteurization.
- *Indirect impact:* Presence of a sufficient level of heat-resistant spoilage enzymes that are not inactivated by pasteurization; raw milk as a source of postpasteurization contamination of pasteurized products in the dairy processing facility.

GENERAL CHARACTERISTICS OF RAW MILK MICROORGANISMS

Thermotolerant Microflora

Microorganisms associated with raw milk may be classified into two categories: thermotolerant bacteria (e.g., survive laboratory pasteurization of 63°C for 30 minutes) and nonthermotolerant bacteria (Bramley and McKinnon, 1990). While certain fungi are heat resistant, most of the heat-resistant microorganisms associated with milk are bacteria, which can be further subdivided into sporulating bacteria and non-sporulating bacteria (Table 5.5).

Table 5.5. Thermotolerant Microbiological Genera Isolated from Raw Milk

Heat-Resistant Sporulating Bacteria	Heat-Resistant Nonsporulating Bacteria
Bacillus	Alcaligenes
Clostridium	Microbacterium
	Micrococcus

Survive heating at 63°C for 30 minutes.

The most heat-resistant bacteria isolated from raw milk are the spore-forming bacteria (e.g., *Bacillus* and *Clostridium*). Spore-forming bacteria are heat resistant in the sporulated states, while germinated vegetative cells (e.g., nonsporulated state) are usually not as heat resistant. If these bacterial spores survive sublethal heat treatment, and if conditions are optimal for the outgrowth of vegetative cells, they can impart spoilage as well as pose food safety risks.

The *Clostridium* spp., being obligate anaerobes, require strict conditions (e.g., absence of oxygen) for growth. Thus, their presence in refrigerated milk and milk products is rare. However, they may exist sporadically in thermally processed canned milk products. The *Bacillus* spp., being aerobic, are more commonly found in milk and milk products than the *Clostridium* spp. As shown in Table 5.4, there are many *Bacillus* spp. that have been isolated from milk or that may be associated with milk and milk products.

The thermotolerant classification for nonsporulating bacteria, may not be completely inclusive, as certain species and strains of the genera listed may be inactivated by heating under the conditions shown (Table 5.4). Further, there are heat-resistant strains of other bacteria (e.g., *Lactobacillus*, *Listeria*, *Mycobacterium*, and *Streptococcus*) that may not be 100% inactivated under these specific conditions, or their inactivation has not been clearly demonstrated.

The relative ratio of thermotolerant and nonthermotolerant bacteria is highly varied and dependent upon the overall quality of the milk (total bacteria count, TBC), season of the year, cow diet and feeding conditions, level of on-farm sanitation, methods used in cleaning and sanitizing, and other variables. For example, in milk with low-standard plate count (SPC; <5,000 CFU/mL), thermotolerant bacteria, especially the *Micrococci*, are often most prevalent. Conversely, nonthermotolerant bacteria, especially *Pseudomonas*, may be more prevalent than thermotolerant bacteria in milk of lower quality or high SPC.

Table 5.6. Psychrotrophic and Nonpsychrotrophic Bacteria Associated with Raw Milk

Psychrotrophic Genera ^a		Nonpsychrotrophic Genera ^b	
<i>Acinetobacter</i>	<i>Flavobacterium</i>	<i>Aerobacter</i>	<i>Leuconostoc</i>
<i>Aerobacter</i>	<i>Listeria</i>	<i>BacillusBrucella</i>	<i>Microbacterium</i>
<i>Aeromonas</i>	<i>Pseudomonas</i>	<i>Clostridium</i>	<i>Micrococcus</i>
<i>Alcaligenes</i>	<i>Yersinia</i>	<i>Coxiella</i>	<i>Salmonella</i>
<i>Athrobacter</i>		<i>Escherichia</i>	<i>Staphylococcus</i>
<i>Bacillus</i>		<i>Lactobacillus</i>	<i>Streptococcus</i>
		<i>Lactococcus</i>	

^aGrowth at 5–7°C in 7–10 days.

^bNo growth at 5–7°C.

Psychrotrophic Microflor

Many of the microorganisms associated with raw milk are psychrotrophs, which are cold-adapted microorganisms with a broad growth temperature range from 0 to +40°C and have an association with habitats that undergo large thermal fluctuation (Guillou and Guespin-Michel, 1996). In milk and milk product testing, psychrotrophs are defined as bacteria demonstrating growth at 5–7°C for 7–10 days (Table 5.6).

It should be noted that psychrotrophic microorganisms grow much more slowly at refrigeration temperatures than at their optimum growth temperature. In addition, there is a considerable variation between, as well as within, the different psychrotrophic genera with regard to growth rate at low temperatures. The most active psychrotrophic bacteria found in milk are Gram-negative rods (GNRs). Certain sporulating bacteria (e.g., *Bacillus*) are also capable of growth under refrigeration at a slower rate. In addition, pathogenic *Listeria monocytogenes* and *Yersinia enterocolitica* are psychrotrophs.

Lactic Acid Bacteria and Related Bacteria

The LAB associated with milk and milk products have been extensively reviewed (Cogan and Accolas, 1990; Tamime, 1990). Some of these bacteria and their characteristics are listed in Table 5.2. While the defining feature of LAB is the fermentation of lactose to lactic acid, there is considerable variation between the LAB in this regard, as different metabolic pathways are used (Cogan and Accolas, 1990). While most of LAB are of farm environment origin, the primary significance is their use as starter cultures in the manufacture of cultured dairy products (e.g., buttermilk, sour cream, cottage cheese, yogurt,

kefi, other products), and cheese. Active dairy starter cultures, selected and maintained by subculturing in milk, are commercially available. The two general types of starter cultures include:

- *Mesophilic Starter Cultures*—optimum growth temperature of approximately 30°C.
- *Thermophilic Starter Cultures*—optimum growth temperature of approximately 45°C.

The LAB are also classified with regard to their fermentation reactions. *Homofermentative* LAB produce lactic acid as the primary fermentation product, while *Heterofermentative* LAB produce other end-products such as acetic acid, carbon dioxide, and other compounds. The LAB also differ in regard to the transport and utilization of lactose, metabolic pathway used for glucose and galactose, as well as proteolysis and amino acid metabolism. In addition, certain types of LAB are used for their metabolism of citrate and flavor production. The LAB used as starter cultures in the dairy industry are discussed below:

- The *Lactococci*: The mesophilic, homofermentative lactococci (e.g., *Lac. lactis* subsp. *lactis*; *Lac. lactis* subsp. *cremoris*) starter cultures are considered the work horses of the dairy industry and are used in the production of a variety of ripened and nonripened cheeses characterized by lower temperature fermentation. The lactococci produce L-lactate as the primary metabolite. In addition, the citrate fermenting *L. lactis* biovar. *diacetylactis* is used in the manufacture of buttermilk, cottage cheese, and sour cream for the production of diacetyl (flavor) and for its antimicrobial activity against *Pseudomonas* spp. and spoilage of cottage cheese.

- The *Leuconostocs*: The common *Leuconostoc* spp. are generally not as active in lactic acid production as the lactococci. Thus, they are usually used as a secondary starter culture. Being heterofermentative, they metabolize lactose to D-lactate, acetate, ethanol, and carbon dioxide. *Leuconostoc mesenteroides* subsp. *cremoris* is used in mixed cultures with lactococci in some cultured dairy products. In addition, *Leu. citrovorum*, which metabolizes citrate to diacetyl, is used for fl vor production in buttermilk, sour cream). Other *Leuconostoc* spp. (e.g., *Leu. mesenteroides* subsp. *mesenteroides*, *Leu. mesenteroides* subsp. *dextranicum*, *Leu. paramesenteroides*, *Leu. lactis*) have been associated with raw milk and dairy products. *Leu. mesenteroides* subsp. *dextranicum* produces extracellular polysaccharide and is of commercial interest for the production of “natural” water-binding stabilizers for use in dairy products.
 - The *Lactobacilli*: There is considerable variation between these *thermophilic* bacteria with regard to lactose metabolism, with certain species and strains being homofermentative and others being heterofermentative. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are the characterizing bacterial culture in yogurt according to the federal standard of identity (21 CFR131.200) and are used as starter cultures in this cultured product. Mixed starter cultures of these two bacteria are also used in the manufacture of cheeses manufactured using higher temperature with high fermentations (e.g., Swiss-type cheeses, Italian cheeses). *Lb. delbrueckii* subsp. *lactis* and *Lb. helveticus* are also used to as starter cultures in high temperature cheese fermentations. These lactobacilli are generally homofermentative, producing D-lactate as the primary metabolite, certain strains of *Lb. lactis* and *Lb. helveticus* are heterofermentative under certain conditions. An extensive group of lactobacilli (and other LAB) are also associated with kefi manufacture.
 - The *Streptococci*: *Streptococcus thermophilus* is the *thermophilic* bacteria used in conjunction with *L. delbrueckii* subsp. *bulgaricus* in yogurt and high temperature cheese fermentations. *St. thermophilus* is homofermentative, but follows different metabolism than lactobacilli and forms L-lactate as the primary metabolite.
 - *Therapeutic LAB*: There are certain LAB strains that have therapeutic or probiotic properties (Holzapfel et al., 1998). These include certain lactobacilli strains (e.g., *L. acidophilus*, *L. casei*) which may colonize the small intestine and *Bifidobacteriu* spp. which may colonize the large intestine. There is considerable industrial interest today in marketing probiotic dairy products. The primary commercial products are yogurt and related fermented products, although many others are available. In addition, milk amended with probiotic cultures is being marketed. Such milk (e.g., acidophilus milk, A-Plus milk, A/B milk, A/B/C milk, and other names) is not fermented, is not sour, and resembles normal milk in fl vor and texture.
 - Other bacteria associated with LAB: *Propionibacterium freudenreichii* subsp. *shermanii* is used as a secondary starter culture in Swiss-type and Dutch-type cheeses. This bacteria ferments lactate to propionate, acetate, and carbon dioxide. The carbon dioxide is responsible for eye formation while propionate and acetate contribute to the nutty/sweet fl vors associated with these cheese varieties. *Brevibacterium linens* is a highly proteolytic bacteria used in surface-ripened cheeses (e.g., Limburger, Brick, Muenster, and related cheese varieties). In traditional manufacture, these bacteria were allowed to colonize the surface from adventitious contamination of the salt brine. However, today many manufactures inoculate the brine or cheese with *Br. linens* cultures.
- Adventitious LAB, from environmental sources in manufacturing plants, may also be involved in the manufacture of cheese, cultured products, and other fermented products, and to, a lesser extent, in butter manufacture. These “wild type” bacteria are often referred to as nonstarter lactic acid bacteria (NSLAB), especially in cheese manufacturing. While a diverse array of bacteria are associated with the NSLAB of certain cheeses. Some examples include:
- *Homofermentative*—*Lactococcus* spp., *Lb. casei*, *Lb. curvatus*, *Lb. plantarum*, *Pediococcus* spp., and others.
 - *Heterofermentative*—*Lb. Fermentum* and others.
- These NSLAB may be associated with characteristic or diversity of fl vors of certain cheese varieties (Fox et al., 1996; Lynch et al., 1997; Steele and Unlu, 1992), but are often associated with a variety of defects described later in this chapter. The association of these adventitious bacteria in raw milk cheese has

been the topic of debate in France, where Registered Designation of Origin (RDO) implies that cheese of specific regions have specific characteristics, related to the breed and nutrition of dairy cows, bacterial microflora and basic cheese manufacturing methods. However, Corroler et al. (1998) examined the genetic diversity of lactococci isolated from raw milk from different Camembert cheese RDOs. They found some diversity between RDOs, but concluded that *L. lactis* subsp. *lactis* is of general farm origin rather than the geographic area.

Coliform Bacteria

The genera associated with the Coliform group include *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* (Guentzel, 2007). Testing for these nonpsychrotrophic bacteria is used as a general indicator of contamination. As some of the coliforms are associated with animal and human feces, a coliform test may also indicate fecal contamination. *Escherichia coli* is the primary pathogen associated with the coliform group.

On-Farm Sources of Contamination and Growth of Microorganisms

Microbiological contamination of milk on the farm can occur from a variety of sources. In addition, other factors (e.g., cooling and storage conditions) will impact the growth of certain microorganisms, and contribute to the level of microorganisms in raw milk. The four main on-farm sources of raw milk contamination (Bramley and McKinnon, 1990; Murphy and Boor, 2000) are the udder interior, the udder exterior, surfaces of milk handling and storage equipment, cleaning and sanitizing procedures, and other environmental sources.

The Udder Interior

It is often assumed that freshly secreted milk, aseptically drawn from the udder of healthy animals, is sterile (Tolle, 1980). However, it has been shown that microorganisms are, in fact, present in aseptically drawn milk from apparently healthy cows through colonization of the teat cistern, teat canal, and teat apex (Kurweil and Busse, 1973). However, it is generally assumed that the udder interior of healthy animals does not contribute significantly to the TBC of bulk tank milk (Murphy and Boor, 2000).

Mastitis is the most significant source of microorganisms in milk which can be related to the udder interior. Animals with either clinical or subclinical mastitis may shed these causative microorganisms into the milk, and depending upon the type of microorganism, and the level of contamination, one infected animal may significantly impact the TBC of bulk tank milk (Bramley and McKinnon, 1990). In addition, spread of mastitis is often dependent upon milking practices. Lactating animals may also be infected with other nonmastitis pathogens (e.g., *Mycobacterium bovis*, *Bru. abortus*, *Coxiella burnetii*) which may be shed into the milk from the udder interior.

As shown in Tables 5.1–5.4, there are several bacterial genera associated with mastitis in lactating animals. The bacterial agents most often associated with mastitis are *Streptococcus agalactiae* and *Str. uberis* (Bramley et al., 1984; Bramley and McKinnon, 1990; Gonzalez et al., 1986; Jeffrey and Wilson, 1987). In a more recent survey by Hayes et al. (2001), *Str. uberis* was the predominant bacteria associated with sudden elevations in bacterial counts (or “spikes”) of bulk tank milk over a 2-week period. Presence of these streptococci in raw milk is a strong indication of infected animals in the herd (Bramley and McKinnon, 1990; Gonzalez et al., 1986). *E. coli*, *Staphylococcus aureus*, and *Mycoplasma bovis* (Gonzalez et al., 1986; González and Sears, 1984) are also associated with mastitis, but to a lesser extent than the streptococci. In mastitis caused by *S. aureus*, the bacteria may colonize the teat duct, and may synthesize and excrete heat stable enterotoxins into the milk (Masud et al., 1993). While *S. aureus* and *E. coli* may contaminate the milk from the udder interior which may contribute to TBC (Gonzalez et al., 1986), their presence in bulk tank milk is more likely to be associated with environmental sources. Sporadic mastitis also occurs by infection of other bacteria such as *Pseudomonas aeruginosa* and *L. monocytogenes* (Moustafa and Marth, 1993) and *B. cereus* (Gonzalez, 1996).

To a lesser extent than the bacterial agents, yeasts (e.g., *Candida albicans*) and nonpigmented algae (e.g., *Prototheca* spp.), may be associated with mastitis (Gonzalez, 1996). Protothecal mastitis infections are primarily caused by *P. zopfi*, while human infections are primarily associated with *P. wickerhamii* (Roesler and Hensel, 2003). *Prototheca* spp. have been isolated from bulk milk samples in Brazil (Costa et al., 1997; Melville et al., 1999) and in Italy (Buzzini et al., 2004), and some strains may survive pasteurization (Melville et al., 1999). While cutaneous and

systemic human infection with *Prototheca* spp. have been reported in patients with compromised immune systems (Melville et al., 1999; Roesler and Hensel, 2003), human protothecal infections are rarely food-borne. At present, the significance of *Prototheca* spp. in raw milk has not been clearly established.

Effective mastitis control programs play a major role in reducing the spread of mastitis microorganisms (Galton et al., 1984). These control procedures include teat dipping and sanitization, milking time sanitation, isolation and separation of infected animals, judicious prophylactic antibiotic treatment, and therapeutic use of antibiotics in dry cows. The somatic cell count (SCC) in raw milk is used as an index of mastitis under the PMO and other regulations. An elevated SCC may serve as an indication of contamination, especially for the *Streptococcus* spp. (Fenlon et al., 1995). In addition to being a concern from a food safety perspective, high SCC also impacts spoilage and quality of dairy products.

While the microorganisms that cause mastitis survive in refrigerated milk, they generally will not grow at temperatures of 10°C or less, except some strains of *S. uberis* (Bramley and McKinnon, 1990). In addition, the mastitis pathogens do not generally survive pasteurization treatment. Thus, if present in pasteurized milk and milk products, it is most probably a result of postpasteurization contamination or inadequate pasteurization.

The Udder Exterior

The teat exterior contains naturally occurring microorganisms which may be shed into the milk. However, the contribution of these microorganisms is considered to be minor (Murphy and Boor, 2000). Of much higher significance is the contamination of the teat exterior from environmental sources (e.g., manure, soil, feed, bedding; Hogan, et al., 1989). While the teat may be contaminated with a diverse array of microorganisms, the most commonly isolated genera include *Micrococcus*, *Bacillus*, *Clostridium*, *Yersinia*, *Aerobacter*, and *Listeria*, as well as certain viruses (Bramley, 1982; Bramley and McKinnon, 1990; Hogan et al., 1989; Zehner et al., 1986). Prevention of contamination of milk from this route is minimized through sanitation practices, especially during milking. Premilking udder hygiene techniques (e.g., thorough cleaning, teat sanitizing, drying) are strongly correlated with the bacteria count of milk (Bramley and McKinnon, 1990; Galton et al., 1984; McKinnon et al., 1990; Pankey, 1989).

Surfaces of Milk Handling and Storage Equipment, and Cleaning and Sanitizing Practices

Improper and inadequate cleaning and sanitizing of milking equipment (e.g., milking machine, bulk tank) is probably the most significant source of environmental contamination of milk (Olson and Mocquat, 1980). Milk residue left on equipment surfaces supports the growth of microorganisms and formation of biofilm which shed bacteria into the milk.

In addition to improper cleaning and sanitizing, equipment of poor sanitary design, or that has not been maintained, contribute to microbial contamination of the milk. Poorly designed and maintained metal surfaces (e.g., stainless steel, welds, and connections) and nonmetal surfaces (e.g., rubber gaskets, milking inflations other surfaces) with cracks and crevices which cannot be adequately cleaned, may enhance biofilm formation. In addition, installations with piping connections that are not substantially flush (creating dead ends), or such that the equipment is not self-draining, may allow pooling and potential incubation and growth of microorganisms. Sanitary design and installation criteria for milking equipment and milk storage vessels are described under 3-A Sanitary Standards (3-A Sanitary Standards, Inc., 2007), which specify the surface materials, finish and fabrication, as well as the construction of the equipment. Finally, if equipment is improperly stored, recontamination with microorganisms may occur, even on adequately cleaned and sanitized equipment.

Other aspects of the cleaning and sanitizing procedures may also impact the TBC and/or select for specific types of microorganisms. For example, more resistant and/or thermotolerant bacteria counts in milk are often associated with use of hot water in cleaning procedures, and readily form biofilm on equipment of poor sanitary design (especially old, cracked rubber surfaces; Murphy and Boor, 2000; Thomas et al., 1966). Conversely, the use of low temperatures and/or insufficient level of chemical sanitizers may select for the psychrotrophic GNRs (Olson and Mocquat, 1980). This is especially the case in inadequately cleaned and sanitized refrigerated storage tanks (MacKenzie, 1973; Thomas, 1974).

Other Environmental Sources

Finally, there are many other sources on the farm that may contribute to microorganisms in milk. Sources of these microorganisms include milking personnel,

environmental sources such as airborne contamination, milking equipment, and water. Historically, with hand milking, the milking personnel had a more significant role in raw milk than today with machine milking. It has, however, been recommended that workers with certain diseases (e.g., Q fever, hepatitis) should be restricted from milking (Griffiths 2000). Airborne contamination during milking should be controlled where feasible, as it is a potential source of milk contamination, especially with spore-forming bacteria (e.g., *Bacillus*, *Clostridium*).

The water used in cleaning and sanitizing as well as other milk production practices is a potential source of contamination with bacteria (e.g., *Pseudomonas*, *Bacillus*, coliforms, other bacteria), viruses, and parasitic protozoa (e.g., *Cryptosporidium*, *Cyclospora*, *Giardia*; Griffiths 2000). The dairy farm water supply should be potable, regularly tested for microbiological contamination, properly located and protected from contamination. Questionable water supplies should be adequately sanitized with an effective, food-grade sanitizer.

Cooling and Storage Practices

In addition to preventing microbial contamination, other factors related to refrigerated storage (e.g., cooling rate, storage temperature, and storage time) are important to minimizing their growth. According to the PMO (PMO, 2005), raw milk for pasteurization shall be “cooled to 10°C (50°F) or less within 4 hours or less, of the commencement of milking, and to 7°C (45°F) or less within 2 hours after the completion of milking,” and “all raw milk and milk products shall be maintained at 7°C (45°F) or less until processed.” In addition, farm bulk tanks shall be equipped with a recording thermometer.

It is a recommended industry practice that milk be stored at lower temperatures than 7°C (45°F) to minimize growth of psychrotrophic microorganisms. According to the investigation by Gehringer (1980), the psychrotrophic population may initially contribute less than 10% to the microflora but can become dominant after 2–3 days at 4.4°C (40°F). Lower temperature storage will delay this shift in the microflora. Improper rate of cooling, and storage of milk at temperatures greater than 7.2°C (45°F) will also allow the growth of certain nonpsychrotrophic bacteria, as well as enhance the growth of the psychrotrophic bacteria. Raw milk for pasteurization may be stored for a maximum of 72 hours prior to processing, and raw milk storage tanks must be emptied,

cleaned, and sanitized every 72 hours (PMO, 2005). While there is some variation depending upon the initial microflora (Bramley and McKinnon, 1990), the levels of streptococci and LAB, are often correlated with improper cooling and storage on the farm (Atherton and Dodge, 1970). This is especially the case for milk that has been stored under temperature abuse conditions such as 15°C (60°F; Gehringer, 1980).

Transportation and subsequent storage of raw milk at the processing facility is a potential source of contamination and/or proliferation of microorganisms. The farm milk hauler is usually responsible for taking samples at the farm for microbiological analyses, and has the authority to reject milk that appears abnormal or is not at 7.2°C (45°F) or below. Inadequately cleaned and sanitized bulk milk tankers and storage vessels are a source of contamination as well. The cleaning of these vessels is primarily done using clean-in-place (CIP) cleaning. The effectiveness of this process is dependent upon properly installed, operated, and maintained CIP equipment as well as routine evaluation of the cleaning process and cleanliness of surfaces (Bell et al., 1994; Winniczuk and Goodrich-Schneider, 2007).

PATHOGENS ASSOCIATED WITH MILK AND MILK PRODUCTS

GENERAL CHARACTERISTICS

The presence of pathogenic microorganisms in raw milk has been well documented (Boor 1997; Bryan, 1983; Donnelly, 1990; Jayarao et al., 2006; Potter et al., 1984; Rohrbach et al., 1992; Van Kessel et al., 2004). In early years, contaminated milk was responsible for the spread of serious diseases, including diphtheria, typhoid, tuberculosis, and brucellosis (Johnson et al., 1990). While improved sanitation and pasteurization innovations have minimized milk-borne infections of these diseases, milk has been the source of other diseases. Foodborne illness outbreaks and food product recalls have been associated with the consumption of raw milk, milk, and milk products that have not been properly pasteurized, and of pasteurized milk and milk products which have been contaminated via postpasteurization (Boor, 1997; Donnelly, 1990; Headrick et al., 1998). A partial listing of pathogens that have been associated with milk and milk products, and the disease with which they are associated, is presented in Table 5.7.

Table 5.7. Pathogens That May Be Associated with Milk and Milk Products, and the Diseases They Cause

Microorganism	Associated Human Diseases and Complications
Bacteria	
<i>Aerobacter aerogenes</i> (<i>Klebsiella pneumoniae</i>)	Urinary and respiratory infections
<i>Bacillus cereus</i>	Invasive infection, gastroenteritis emetic intoxication
<i>Brucella abortus</i>	Undulant fever
<i>Campylobacter jejuni</i>	Invasive infection, gastroenteritis, Guillan–Barre syndrome, colitis, septicemia
<i>Clostridium perfringens</i>	Invasive infection, gastroenteritis
<i>Corynebacterium</i> spp.	Diphtheria
<i>Coxiella burnetii</i>	Q fever
<i>E. coli</i> —nonverotoxigenic	Invasive infections, gastroenteritis
<i>E. coli</i> —verotoxigenic or enterohemorrhagic)	Toxicoinfection, hemolytic uremic syndrome (children); TPP (elderly)
O157:H7 and others	
<i>Listeria monocytogenes</i>	Listeriosis
<i>Mycobacterium bovis</i> , mycotuberculosis	Tuberculosis
<i>Myco. avium</i> subsp. <i>paratuberculosis</i>	Crohn’s disease (possible link)
<i>Pseudomonas aeruginosa</i>	Invasive infection, gastroenteritis
<i>Salmonella</i> spp.	Invasive infection, gastroenteritis
<i>Staphylococcus aureus</i>	Emetic intoxication
<i>Streptococcus agalactiae</i>	Sore throat
<i>Str. Pyogenes</i>	Scarlet fever/sore throat
<i>Str. zooepidermicus</i>	Pharyngitis, nephritic sequelae
<i>Yersinia enterocolitica</i>	Invasive infection, gastroenteritis
Viral agents	
Enterovirus, rotavirus, coxsackie virus	Invasive infection, gastroenteritis
Hepatitis viruses	Infectious hepatitis
Parasitic protozoa	
<i>Cryptosporidium parvum</i>	Invasive infection, gastroenteritis
<i>Entamoeba histolytica</i>	Amebiasis
<i>Giardia lamblia</i>	Giardiasis
<i>Toxoplasma gondii</i>	Toxoplasmosis

The majority of milk-borne illness outbreaks are associated with consumption of raw or improperly pasteurized milk and milk products. According to the survey report by the National Association of State Departments of Agriculture (NASDA) in 2004 (NASDA, 2004), 29 states have recorded illness outbreaks traceable to raw milk consumption. Further, in 2005–2006, more than 10 outbreaks caused by the consumption of raw milk or raw milk cheese were reported by the Food and Drug Administration (FDA, 2006).

The recent survey by Jayarao et al. (2006) identifies several foodborne pathogenic bacteria associated with raw milk, including *Campylobacter jejuni*, Shigatoxin producing *E. coli*, *L. monocy-*

togenes, *Salmonella* serovars, and *Y. enterocolitica*. In addition, unpasteurized milk is a vehicle for transmission of other pathogenic bacteria (e.g., *Brucella*, *Mycobacterium*, *Staphylococcus*, *Streptococcus*), viruses (e.g., *Hepatitis A*), and “Brainerd Diarrhea” (FDA, 2007; Headrick et al., 1998). While milk-borne pathogens can affect the health of anyone who drinks raw milk, they are especially dangerous to high-risk consumers (e.g., pregnant women, children, the elderly, and people with weakened immune systems).

In recent years, the primary causative agents of milk-borne illness are *C. jejuni*, *Salmonella* spp., *L. monocytogenes*, and *Y. enterocolitica* (Griffiths 2000). Other bacteria associated with sporadic

foodborne illness from milk and milk products include *E. coli*, *Staphylococcus aureus*, and *Enterobacter sakazakii*.

Genera *Campylobacter* and *Yersinia*

Reported milk-borne illnesses caused by *C. jejuni* and *C. coli* have almost exclusively been associated with raw milk consumption or inadequate pasteurization (Boor, 1997; CAST, 1994; Potter et al., 1984). *Campylobacter* spp. are not psychrotrophic and are readily inactivated by pasteurization of milk.

Y. enterocolitica has also been associated with consumption of raw or inadequately pasteurized milk products, but to a lesser extent than *Campylobacter* spp. Although certain *Yersinia* spp. cause mastitis, there is a relatively low incidence of human pathogenic strains of *Y. enterocolitica* in the raw milk supply (Byrne, 2004). In 1982, a large outbreak of yersiniosis in pasteurized milk was traced to contamination of milk crates from a hog farm. *Y. enterocolitica* is psychrotrophic and capable of growth under refrigeration temperatures.

Escherichia coli

Several types of *E. coli* (e.g., enteroinvasive, enteropathogenic, enterotoxigenic, and enterohemorrhagic) have been isolated from raw milk. The foodborne illness symptoms associated with *E. coli* infections range from mild gastroenteritis to more serious complications, especially in high-risk individuals. Invasive food infection caused by enteroinvasive and enteropathogenic *E. coli* is usually mild and is usually not considered significant in the United States (CAST, 1994). Illness caused by enterotoxigenic and enterohemorrhagic *E. coli* is termed toxicoinfection, in that these bacteria are noninvasive and cause illness by producing toxins while growing in human intestines. Enterotoxigenic *E. coli* is considered the primary cause of "travelers' diarrhea" and is only a sporadic cause of illness in the United States. Enterohemorrhagic *E. coli* (e.g., *E. coli* O157:H7 and other verotoxigenic *E. coli*), due to severity in high-risk individuals, is a serious foodborne illness agent. The verocytotoxin (e.g., Shiga-like toxin) produced by *E. coli* O157:H7 is associated with hemorrhagic colitis, hemolytic uremic syndrome (kidney failure in children), and thrombotic thrombocytopenic purpura (a brain condition in elderly patients; CAST, 1994). Since *E. coli* are readily destroyed by pasteurization, their presence in processed milk products is the re-

sult of inadequate pasteurization, or postpasteurization contamination. In addition, *E. coli* are generally not psychrotrophic. Thus, invasive *E. coli* infections often involve storage at improper temperatures that allow growth to reach a sufficient level to cause illness. However, disease caused by *E. coli* O157:H7, being a toxicoinfection, may occur at a very low infective dose depending upon the host (CAST, 1994). *E. coli* O157:H7 outbreaks have been associated with raw milk, cheese curds from milk that was inadequately pasteurized, and in cheese via postpasteurization contamination.

Listeria monocytogenes

Listeria monocytogenes, its characteristics and relationship to food safety, has been extensively reviewed (Ryser and Marth, 1991). Human listeriosis, caused by ingestion of *L. monocytogenes*, is a serious foodborne illness that may be associated with abortion in pregnant women, and meningitis, encephalitis, and septicemia in newborn infants and immunocompromised adults. In addition, outbreaks involving *L. monocytogenes* are characterized by a comparatively high mortality rate (CAST, 1994). Adults in the low-risk category are virtually unaffected by *L. monocytogenes*. Because this bacteria is an animal pathogen, the route of infection in raw milk is often via the shedding the bacteria into the udder. Even mildly infected or apparently healthy animals may shed these bacteria into the milk (Byrne, 2004). *L. monocytogenes* is also associated with damp environments and may enter raw milk or pasteurized milk and milk products through aerosolization on the farm or in the processing facility. Being an active psychrotroph, *L. monocytogenes* is capable of growth under refrigerated conditions.

L. monocytogenes is more heat resistant than other pathogens. However, it is inactivated by pasteurization treatments currently applied in the United States. *L. monocytogenes* has been sporadically associated with raw milk, soft-ripened cheeses, and other dairy products. The most high-profile outbreak in the United States occurred in 1985 and was associated with consumption of Mexican-style cheese products (discussed further below).

In 2003, FDA, USDA/Food Safety and Inspection Service (FSIS), and Centers for Disease Control and Prevention (CDC) published a collaborative *L. monocytogenes* risk assessment (Anonymous, 2003b). The dairy products listed in the higher risk category in this risk assessment include raw (unpasteurized) milk,

and soft and unripened cheeses made from raw (unpasteurized) milk. The FDA has also published recommendations for consumers to avoid food-related illness from *L. monocytogenes*. The following are the recommendations related to dairy products:

- Do not eat soft cheese such as Feta, Brie, and Camembert cheeses, blue-veined cheeses, queso blanco, queso fresco, and Panela unless it is labeled as made with pasteurized milk.
- Do not drink raw (unpasteurized) milk or eat foods that contain unpasteurized milk.

Genus *Salmonella*

The incidence of *Salmonella* spp. in raw bulk tank milk has been estimated at 4.7% (Byrne, 2004) and this genus has been associated with milk-borne infections from consumption of raw milk. Because *Salmonella* spp. are readily destroyed by pasteurization, salmonellosis from pasteurized milk products is primarily due to inadequate pasteurization or postpasteurization contamination (Read et al., 1968). In general, *Salmonella* spp. are not psychrotrophic, although certain strains may grow very slowly under refrigeration (CAST, 1994). In addition, *Salmonella* spp. have the ability to survive for long periods of time in dehydrated food products (e.g., dry milk products, cocoa products, dry ingredients; Juvan et al., 1984). In the 1960s, outbreaks of salmonellosis were associated with dry milk products. In 1982, *S. typhimurium* was implicated in a large Canadian outbreak attributed to inadequate pasteurization (Boor, 1997; Donnelly, 1990). Two more recent large, multistate outbreaks of salmonellosis associated with consumption of contaminated pasteurized milk and of ice cream occurred in the United States. The impact of salmonellosis outbreaks on dairy regulations and industry practices is discussed later.

Staphylococcus aureus

Staphylococcal food poisoning is a classical food-borne intoxication, whereby the bacteria reach sufficient levels in food products producing a potent toxin in the food, and the toxin imparts illness upon consumption. Food intoxications are characterized by more rapid onset than foodborne infections. While milk products have been a historical source of staphylococcal food poisoning, improvements in dairy sanitation have minimized the risk of this illness in milk products.

S. aureus is associated with animal wastes, human skin, and is fairly ubiquitous on dairy farm environment. The bacteria itself is not heat stable and is readily inactivated by pasteurization. However, the staphylococcal enterotoxin is highly stable to heating (e.g., not inactivated by pasteurization), and can persist in an active state for long periods in dairy products (e.g., dry milk, ice cream). In addition, *S. aureus* is not psychrotrophic. Therefore, growth of *S. aureus* is readily controlled by adequate refrigeration. However, the bacteria can grow under temperature abuse above 10°C (50°F). *S. aureus* is also salt tolerant.

Because of human contact, exposure to warm fermentation temperatures for long time periods, and salt levels often associated with cheese manufacture, certain cheeses are often considered to be at risk for staphylococcal food poisoning. Contributing factors include inadequate pasteurization, inadequate sanitation, and poor starter culture growth and activity.

Enterobacter sakazakii

Enterobacter sakazakii (formerly “yellow pigmented” *E. cloacae*) has been associated with severe and life-threatening illnesses (e.g., meningitis, bacteremia, necrotizing enterocolitis), especially in neonates and infants (Biering et al., 1989; Bowen and Braden, 2006). While the incidence of food-borne illness from *E. sakazakii* is low, the mortality rate (50–75%), consequences, and severity are high (Nazarowec-White and Farber, 1997a). While the source of these bacteria has not been clearly established, it has been associated with powdered infant formulae, especially in neonatal intensive care situations (CDC, 2002; Simmons et al., 1989; van Acker et al., 2001), and powdered milk (Biering et al., 1989). The risk of infection in the hospital intensive care setting is from both intrinsic (from the infant formulae) (Simmons et al., 1989) and extrinsic (nosocomial) factors (Noriega et al., 1990). According to a risk assessment profile conducted by the Codex Committee on Food Hygiene (Codex, 2004):

- *E. sakazakii* is an emerging pathogen linked to the consumption of powdered infant formula with a high risk of potential fatal infection for neonates in hospital settings.
- Factors that contribute to the risk and need more research include level of contamination, thermal stability, rate of growth, infectious dose, and virulence of the pathogen.

E. sakazakii is one of the most thermotolerant bacteria associated with dehydrated infant formula (Nazarowec-White and Farber, 1997b). Survival of *E. sakazakii* in infant formulae has been shown to be effected by water activity (a_w) and temperature, with higher survival at low a_w (0.25–0.30) and low temperature of 4°C (39.2°F; Gurtler and Beuchat, 2007). Although this pathogen and its association with dry milk formulations needs further investigation, the importance of using aseptic methods and proper temperature control in the preparation, use, and storage of dried infant formula is being stressed by regulatory officials, hospitals, and infant formula industry.

IMPACT OF FOODBORNE ILLNESS ON DAIRY INDUSTRY PRACTICES AND REGULATIONS

Historically, foodborne outbreaks associated with milk and milk products have had a direct or indirect impact on dairy regulations as well as industry practices. For example, mandatory milk pasteurization was directly attributed to prevalence of milk-borne illnesses in the early 1900s. There have been significant outbreaks in recent history that have impacted dairy regulatory and/or industry practices as well. These are chronologically discussed below:

- **1960s.** By the mid-1960s, dry milk products had been involved in sporadic outbreaks of salmonellosis in the United States and other parts of the world. In late 1965, a significant multistate outbreak, associated with instant nonfat dry milk contaminated with *S. Newfoundland*, occurred in the United States (Collins et al., 1968). Epidemiological investigation revealed that the apparent cause of the contamination was insufficient filtration of the incoming air intakes for the drying and agglomeration process. Further, inadequate sanitary design of the plant equipment contributed to the contamination. As a result of this outbreak, regulatory surveillance of this industry segment was increased and the U.S. Department of Agriculture (USDA) embarked on a *Salmonella* testing program for dry milk products. The industry responded by implementing improvements in sanitary design of equipment and facilities, with improved air intake systems. In addition, this outbreak stimulated research interest by the scientific community and

the knowledge of the survival characteristics of *Salmonella* has increased considerably since the 1960s. The number of outbreaks in dry milk products has decreased due to these efforts.

- **1980s.** In 1985, two foodborne illness outbreaks occurred in dairy products that had major impact on the dairy industry and its regulation. In March/April 1985, a large multistate outbreak occurred in the Midwest as a result of consumption of pasteurized milk products contaminated with *S. typhimurium*. The milk was produced in a modern, highly automated plant in suburban Chicago, IL. Epidemiological investigation indicated that an engineering problem in the plant allowed the cross-contamination of pasteurized milk with raw milk, and incubation at unsafe temperatures. In June 1985, a listeriosis outbreak was attributed to consumption of Mexican-style cheese contaminated with *L. monocytogenes* in Los Angeles County, CA. Epidemiological investigation indicated poor sanitation conditions as well as inadequate pasteurization or use of raw milk in cheese manufacture. The regulatory response to these two outbreaks by the FDA was the initiation of what became known as the Dairy Safety Initiatives program (Kozak, 1986; Lecos, 1986). These landmark initiatives involved program evaluation, expanded inspection programs which included more evaluation of process design and automation, and a pathogen testing program of dairy products and dairy processing environments. The initial impact was that, due to the finding of pathogens (especially *L. monocytogenes*) in the environment of facilities as well as products, there was an increase in voluntary recalls of dairy products during the late 1980s. In subsequent years, the FDA embarked on pathogen testing and evaluation programs in other food industry segments. In addition, U.S. Department of Agriculture (USDA)/Food Safety and Inspection Service (FSIS) have embraced pathogen testing in regulation of the meat and poultry industries. Because of its association with dairy cattle infections, *L. monocytogenes* was initially characterized to be a “dairy pathogen.” However, it is now well known that *L. monocytogenes* is an environmental microorganism that associates with the cool, damp environments with relevance to nearly every segment of the food processing and handling system where postprocessing

contamination is an issue. Since the 1980s, there has been a food industry-wide effort for improvements in environmentally sanitation practices, which may involve routine testing for the microorganism. Because *L. monocytogenes* generally inhabits similar environments as *Pseudomonas* spp. and other psychrotrophic bacteria, these improved environmental sanitation practices also has improved the shelf life of milk and milk products, and other food products.

- **1990s.** In 1994, another large salmonellosis outbreak was attributed to dairy products and involved a nationwide, door-to-door ice cream distribution. In this outbreak, *S. enteritidis* contaminated pasteurized ice cream mix during transportation in tankers that had previously hauled liquid raw egg products, and had not been adequately cleaned (Hennessy et al., 1996). This outbreak, which had an estimated 224,000 cases of *S. enteritidis* gastroenteritis, is the largest common vehicle outbreak ever recognized in the United States. The result has been heightened awareness, by both the regulatory and industry sector, to concerns with regard to the transportation industry, the adequacy of cleaning procedures, and product safety.
- **2000s.** In 2005, verotoxigenic *E. coli* O157:H7 was implicated in a serious outbreak from consumption of raw milk in Washington and Oregon (CDC, 2007). The farm in Cowlitz County, WA, participated in a cow-share program, whereby customers purchase interests in (or shares of) dairy cows in return for a portion of the milk produced. Cow-share programs are controversial and are illegal in many states. This outbreak and litigation related to the outbreak, has received considerable news media attention, and may be responsible for bringing about changes in state regulatory programs with stricter controls on the sale of raw milk for direct consumption.

IMPACT OF MICROORGANISMS ON THE QUALITY AND SPOILAGE OF MILK AND MILK PRODUCTS

GENERAL ENZYMATIC REACTIONS

The spoilage microorganisms in milk and milk products cause spoilage or quality defects in milk and milk products primarily due to their saccharolytic, proteolytic, and lipolytic activity, and associated enzymes

which impact the flavor and texture of milk and milk products. These reactions are generally discussed as follows.

Saccharolytic Reactions

Saccharolytic reactions involve degrading carbohydrates to form simple sugars (e.g., glucose) followed by subsequent fermentation reactions. Milk sugar (e.g., lactose) is the primary substrate, while other sugars and carbohydrates, when present, also are utilized to a varied degree by these microorganisms. Lactic acid fermentation and related reactions have been discussed previously. Other organic acid and fatty acids are also produced during carbohydrate fermentation, which may be considered desirable or considered undesirable, depending upon the level and type of dairy product. Ethanol fermentation (e.g., ethanol, carbon dioxide) is usually considered undesirable. A notable exception is kefir and related products, where some ethanol fermentation is desirable.

Proteolytic Reactions

Of the milk proteins, the caseins are the most significant substrates for proteolysis, while to a varied degree other proteins may be involved. The enzymes involved are generally classed as *proteinases* (e.g., *endopeptidases*) with active sites in the interior of the polypeptide chain, yielding peptides as the primary product, and *exopeptidases* (e.g., aminopeptidases, carboxypeptidases) with active sites at the ends of the polypeptide chain, yielding amino acids as the primary product. In addition, microorganisms have metabolic enzymes (e.g., transaminases, deaminases) that will further utilize amino acids forming a variety of compounds. The off-flavors and odors associated with proteolytic reactions are bitter, putrid, certain fruity flavors, and other off-flavors. Texture problems associated with proteolysis include

- gelation, coagulation, and sweet-curdling of fluid milk;
- “floatin curd” defect and gelatinous and slimy curd in cottage cheese and related texture problems in other cultured products;
- reduced yield and reduced processing efficiency in cheese production; and
- “open texture” defect of hard cheese varieties.

Lipolytic Reactions

The primary spoilage of milk and milk products due to lipolytic reactions is an off-flavor reaction termed hydrolytic rancidity, or the release of short chain volatile fatty acids from fat molecules. However, lipolysis is also the cause of fruity/fermented flavors in milk and cottage cheese. While endogenous milk lipase plays a role in hydrolytic rancidity in raw and unpasteurized milk, microbial lipases are the major contributor to lipolytic spoilage and hydrolytic rancidity in pasteurized milk and milk products.

IMPACT OF SPECIFIC CLASSES OF MICROORGANISMS ON QUALITY AND SPOILAGE

Mastitis Bacteria and Somatic Cells

High SCC negatively alters milk yield, composition, processing properties, and the yield and quality of milk products (Munro et al., 1984) and has also been associated with increased proteolytic and lipolytic activities (Azzara and Dimmick, 1985; Verdi and Barbano, 1988). Decreased shelf life of pasteurized milk has been associated with high SCC in the raw milk (Janzen, 1985; Ma et al., 2000; Rogers and Mitchell, 1989). The impacts of high SCC in cheese milk are reduced curd firmness, decreased yield, and lowered sensory quality of cheese products (Barbano et al., 1991; Klei et al., 1998; Politis and Ng-Kwai-Hang, 1988).

Mastitis and Coliform Bacteria

If present in pasteurized milk some of the mastitis causing microorganisms may cause spoilage through proteolytic and lipolytic activity (Azzara and Dimmick, 1985). In addition, high SCC count has been associated with a variety of quality-related defects in milk and milk products such as poor rennet action and decreased cheese yields (Barbano et al., 1991).

The coliform bacteria are often associated with unclean flavors in cultured dairy products and cheese products. In addition, high coliform count is often associated with excessive gas formation (“early blowing”) in ripened cheese (Mullan, 2003).

Lactic Acid Bacteria and Relationship to Quality Defects

Lactic acid fermentation reactions, if appropriately optimized and balanced, are desirable in cultured

products, cheese products, and other fermented products. These fermentation reactions, in fact, define the characteristics of these products. However, lactic acid formation is undesirable when associated with fluid milk and unfermented dairy products. The primary spoilage consequence of lactic acid fermentation is souring and coagulation. Other fermentation products may be associated with undesirable flavors. LAB and related bacteria are not psychrotrophic, thus souring of milk and associated elevated titratable acidity (TA) is rare in adequately refrigerated milk. Thus, elevated TA is an indication of temperature abuse on the farm or during transportation. In the past, the TA test was routinely done on incoming raw milk at the processing facilities or receiving stations. However, due to more efficient and more controlled refrigeration, the TA test is not done routinely today.

The LAB starter cultures themselves may be associated with quality degradation in cultured products as well as cheese products, if inappropriately selected, or if conditions of growth are improper or not optimum. Some examples are discussed below:

- *Cultured Dairy Products:* A variety of quality defects in cultured dairy products are associated with overactive, underactive, or inappropriately selected LAB starter cultures (Moore and Wilson, 2007). Some of the more common flavor defects associated with starter culture activity include bitterness (proteolytic strains), high acid (overactive lactic acid production), low acid (underactive lactic acid production), and high diacetyl (overactive diacetyl culture). Flavor defects (e.g., unclean, rancid) may also be related to starter cultures, but are more commonly associated with other microorganisms.
- *Yogurt:* For high-quality yogurt, care should be used in selecting starter culture strains and an appropriate balance of *S. thermophilus* and *L. delbreuckii* subsp. *bulgaricus* should be maintained, as overgrowth of either culture can result in flavor and texture defects in the yogurt. A lactobacilli to streptococci ratio of approximately 1:1 is usually considered desirable. Overgrowth of *S. thermophilus* is usually associated with bland, less acid, and less firm yogurt. Conversely, overgrowth of the lactobacilli results in high acid, more firm yogurt, which may also have bitter flavor due to peptides formed from proteolysis. Higher level of D-lactate from the lactobacilli is also associated with bitter flavor. The relationship

between these two cultures has been characterized as “symbiotic” (although proto-cooperation may be the more appropriate term) where they stimulate each other with respect to growth as well as the production of typical “green apple” yogurt flavor, due primarily to acetaldehyde. While acetaldehyde is a metabolic product of carbohydrate metabolism, a portion is synthesized from the amino acid, threonine, via threonine aldolase. The threonine aldolase pathway may account for increased acetaldehyde in mixed culture milk fermentations. In general, the lactobacilli, being more proteolytic, provide peptides and amino acids for stimulated growth and acetaldehyde synthesis by the streptococci (Wilkins et al., 1986). Yogurt fermented with *L. acidophilus* tends to have lower yogurt flavor primarily due to alcohol dehydrogenase activity of *L. acidophilus* strains, which degrades the acetaldehyde over time.

- **Cheese Products:** The role of the LAB starter culture in the complex proteolytic scheme of ripened cheese is well documented (Fox et al., 2000; Lynch et al., 1997; Steele and Unlu, 1992). In addition, LAB peptidases also have been related to bitter peptide formation in ripened cheeses. A variety of quality defects (e.g., high acidity, gassiness, and volatile off-flavors) in cheese and related products are attributed to NSLAB. The NSLAB are also considered to be a cause of calcium lactate crystallization on the surface of Cheddar cheese (Chou et al., 2003).

THE GRAM-NEGATIVE RODS

The primary GNR bacteria associated with milk and milk products which are involved in spoilage include *Pseudomonas*, *Enterobacteriaceae*, *Alcaligenes*, *Acinetobacter*, *Chromobacterium*, and *Flavobacterium* (Cousin, 1982; Craven and Macauley, 1992a,b,c; Uraz and Citak, 1999). As indicated in Table 5.1, many of these genera are psychrotrophic and capable of growth under refrigeration. With the exception of certain *Alcaligenes* spp., these bacteria are not thermophilic and do not survive normal pasteurization. Thus, they are usually postpasteurization contaminants in pasteurized dairy products. There is some variation as to the spoilage impact of these bacteria. Some of these genera are proteolytic and/or lipolytic and are associated with spoilage reactions imparting texture and flavor problems associated with enzymatic hydrolysis of proteins and

fat. In general, the GNRs are not active carbohydrate fermenters and usually do not produce lactic acid.

Psychrotrophic GNRs have been identified as the primary contaminants which impact the shelf life of conventionally pasteurized milk and milk products (Cromie, 1991; Gruetzmacher and Bradley, 1999), especially via contamination at the filling machine. Strict attention to environmental sanitation has resulted in dramatically decreased the level of contamination and has resulted in increased shelf life of refrigerated milk and milk products.

The major GNR associated with pasteurized milk products are *Pseudomonas* (e.g., *Ps. fluorescens*, *Ps. fragi*, *Ps. putida*; Cousin, 1982; Craven and Macauley, 1992a,b,c). *Ps. fluorescens* is considered to be a primary source of proteolytic spoilage of many refrigerated dairy products including milk, cottage cheese, yogurt, and other cultured products (Bigalke, 1985a). *Ps. fluorescens* has also been identified as a major contributor to increased free fatty acid and rancidity in fluid milk (Fitz-Gerald and Deeth, 1983). *Ps. fragi* is often associated with lipolytic off-flavors in milk products, as well as proteolytic reactions and slime formation in cottage cheese (Marth, 1970). *Pseudomonas* spp. and other psychrotrophic spoilage bacteria are also responsible for degradation of diacetyl via diacetyl reductase and flavor loss in buttermilk (Wang and Frank, 1981).

The proteinases and lipases extrapolated by *Ps. fluorescens* and *Ps. fragi* are highly heat-stable and are not inactivated by pasteurization nor ultrahigh temperature (UHT) sterilization processes (Bigalke, 1985b; Fairbairn and Law, 1986; Law, 1979; Law et al., 1976). The association of the highly heat-resistant *Ps. fluorescens* proteinases with bitter flavor formation and gelation of UHT milk and cream products has been well documented (Law et al., 1977). Through improved sanitation and minimizing the practice of pooling and storing milk and cream prior to sterilization has greatly minimized these spoilage defects in UHT products.

Thermophilic Spore-forming Bacteria and Spoilage

As shown in Table 5.5, Gram-positive spore-forming microorganisms are thermophilic and survive pasteurization. Following pasteurization, if conditions allow germination and growth of vegetative cells, these bacteria may cause spoilage of milk products. In addition, they may contaminate milk products via the postpasteurization route.

Clostridia are strict anaerobes and only certain strains are capable of psychrotrophic growth. Thus, *Clostridium* spp. are only sporadically implicated in spoilage of milk and milk products. However, many *Clostridium* spp. are saccharolytic, proteolytic, and/or lipolytic. Thus, if conditions are conducive to their growth, they can cause spoilage, usually with gas formation. For example, *Cl. butyricum*, *Cl. tyrobutyricum*, and *Cl. sporogenes* may be associated with “late blowing defect” of cheeses, as well as off-flavors associated with butyric acid, acetic acid, ammonia, and hydrogen sulfide (Klijn et al., 1995).

Many of the *Bacillus* are proteolytic, lipolytic, and saccharolytic and capable of causing spoilage if allowed to grow to sufficient level. Thus, *B. stearothermophilus* may be associated with spoilage of canned milk products stored at room temperature. However, in refrigerated milk and milk products, the *Bacillus* spp. capable of psychrotrophic growth (e.g., *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. pumilus*, *B. subtilis*) are more significant (Meer et al., 1991). Since these bacteria grow more slowly than *Pseudomonas* spp., they have historically not been considered of consequence with regard to reducing shelf life of conventionally pasteurized milk products. However, Fromm and Boor (2004) have recently identified heat-resistant, psychrotrophic Gram-positive rods (e.g., *Paenibacillus*, *Bacillus*, *Microbacterium*) as predominant microorganisms in pasteurized milk and suggested that these bacteria may be the next hurdle to overcome in further extending the shelf life of pasteurized milk products.

Yeasts and Spoilage of Dairy Products

As shown in Table 5.4, many yeast genera are used in the manufacture of kefir and are often used in mixed kefir starter cultures in this highly variable fermented dairy product. Yeasts may also contribute to characteristic flavors of some cheese products via airborne contamination (Lopandic et al., 2006). These include *Debaryomyces hansenii*, *Candida* spp., and *Kluyveromyces marxianus* var. *lactis*.

The majority of yeast spoilage of dairy products is via postpasteurization contamination, as yeasts are ubiquitous airborne contaminants in many processing and storage facilities. Yeasts are also associated with the brine of Feta and related cheese products (Kaminarides and Laskos, 1992).

The most characteristic spoilage caused by yeast is the result of ethanol fermentation reactions resulting in “fermented” off-flavors and “gassy spoilage.” Cer-

tain yeast genera are proteolytic and lipolytic and are associated with these enzyme reactions in milk and milk product spoilage. Yeasts contaminate nearly all dairy products, but the consequence may be most noticeable in yogurt and other cultured dairy products (Yamani and Abu-Jaber, 1994).

Heat-resistant yeasts are important spoilage microorganisms in many aseptic fruit juice products, but are rarely associated with dairy products. Heat-resistant *Candida lacticondensi* has been shown to cause occasional spoilage of sweetened condensed milk (Gilmour and Rowe, 1990).

Mold and Spoilage of Dairy Products

Certain molds are used in the manufacture of cheese products. Blue-green molds (e.g., *Penicillium roqueforti*, *Pen. glaucum*) are used as cultures in the manufacture of blue veined, interior ripened cheese varieties and, primarily through lipolysis, are responsible for the characteristic flavor or blue-green veining of these cheeses. *Pen. casei*, a yellowish-brown mold, is thought to be associated with the ripening of Swiss-type cheeses via airborne contamination. Grey and white molds (e.g., *Pen. album*, *Pen. camemberti*, *Pen. candida*, and *Pen. caseicola*) are used in the manufacture of surface ripened, soft cheeses such as camembert and brie. These molds are generally proteolytic in nature, and are responsible for the characteristic flavors as well as the “softening” of the texture. *Geotrichum candidum* (“machinery mold”) is also associated with these surface-ripened cheeses, but usually as an airborne contaminant (Marcellino et al., 2001).

Certain molds produce mycotoxins, which are liver carcinogens. For example, aflatoxin M1 has been isolated from raw milk from cows which have been fed corn contaminated with *Aspergillus flavus*. Therefore, the FDA has established an action guideline of 0.5 ppb for aflatoxin M1 in milk (FDA, 2005). The FDA, as well as the dairy industry, conducts routine aflatoxin monitoring of raw milk. *Aspergillus* spp. are not usually airborne contaminants in dairy processing and handling facilities.

Spoilage of dairy products by molds is usually due to airborne, postpasteurization contamination. While mold is usually not involved with spoilage of fluid milk and fluid milk products, it may cause off-flavors in some cultured products. The highest consequence of mold spoilage is in cheese and butter products where mold growth is associated with musty/bitter flavors. Certain highly proteolytic molds (e.g., *Scopulariopsis brevicaulis*) cause

Table 5.8. Microbiological Standards for Raw and Pasteurized Milk According to Regulations in the United States, Canada, EEC, and Australia/New Zealand

Country	Raw Milk (Producer)		Pasteurized Milk	
	TBC ^a	SCC ^b	TBC	CC ^c
USA ^d	100,000	750,000	20,000	10
Canada ^e	50,000	500,000	$m = 10,000$ $M = 25,000$	$m = 1$ $M = 10$
EEC ^e	100,000	400,000	$m = 50,000^f$ $M = 500,000^f$	$m = 0^f$ $M = 5^f$
Australia/New Zealand ^e	150,000		$m = 50,000$ $M = 100,000$	$m = 1$ $M = 10$

Adapted from Anonymous (2003a, b).

^a TBC, total bacteria count (as measured by standard plate count or equivalent) as CFU/mL or g.

^b SCC, somatic cell count.

^c CC, coliform count.

^d Upper limit for Grade A milk, regulated under the Pasteurized Milk Ordinance (PMO).

^e m, maximum TBC that is of no concern or acceptable level of contamination; M, maximum TBC, that if exceeded by any one sample unit renders the lot in violation of the regulation).

^f After incubation for 5 days at 6°C.

sporadic spoilage of surface-ripened cheeses which is associated with ammonia production. Heat-resistant molds (e.g., *Sporendonema sebi*) may cause spoilage of sweetened condensed milk, though this type of spoilage is rare.

Interactions of Microorganisms in Milk and Milk Products

It has been well documented that LAB inhibit the growth of competing microorganisms to a varied degree (Cogan and Accolas, 1990). This inhibition is due to synthesis of lactic acid (reduced pH), organic acids, synthesis, and antimicrobial compounds (bacteriocins and other compounds), physiological and other interactions between microorganisms in milk have not been extensively investigated.

Prior growth of *Pseudomonas* spp. has been shown to stimulate the growth of other microorganisms in milk including yogurt starter cultures and LAB starter cultures (Cousin and Marth, 1977). In addition, *Pseudomonas* spp. and *Achromobacter* may stimulate the growth of *Staph. aureus* in milk (Seminiano and Frazer, 1966). Antagonism between *Pseudomonas* spp. and other microorganisms has also been shown (Freedman et al., 1989).

The interactions between *L. monocytogenes* and other psychrotrophic bacteria in milk and milk products have been reviewed (Frag and Marth, 1992). Initial investigations (Marshall and Schmidt, 1988)

indicated that *L. monocytogenes* grew competitively with *Ps. fragi* but its growth at 10°C was neither stimulated nor inhibited. However, preincubation of milk at 10°C (50°F) with either *Ps. fragi* or *Ps. fluo escens*, stimulated the growth of *L. monocytogenes* (Marshall and Schmidt, 1988). These results were corroborated by Farag and Marth (1989) at different temperatures, whereby preincubation with *Ps. fluo escens* stimulated growth of *L. monocytogenes* at 7°C (44.6°F) and at 13°C (55.4°F). However, the growth of *L. monocytogenes* decreased slightly between 14 and 56 days. A mechanism of stimulated *L. monocytogenes* in milk was shown to be due to proteolysis (Marshall and Schmidt, 1991). Stimulated growth of *L. monocytogenes* has also been related to proteolytic reactions in Camembert and other soft cheeses (Back et al., 1993).

MICROBIOLOGICAL ANALYSES OF MILK AND MILK PRODUCTS

Microbiological Standards

A comparison of microbiological standards for the United States, Canada, EEC, and Australia/New Zealand (Anonymous, 2003a) is presented in Table 5.8.

For the United States, the data shown are the upper limits of TBC, SCC, and coliform count (CC) for Grade A milk (PMO, 2005). For the other countries listed, microbiological standards for pasteurized milk

are intricately based upon the number of samples (n) and TBC limits are two-tiered as shown. The USDA recommended standards for milk for manufacturing purpose (e.g., non-Grade A milk) specify that such milk be rejected if 3 out of the last 5 samples has a TBC of 500,000 CFU/mL or greater and/or SCC count of 750,000 CFU/mL or greater (USDA, 2007).

Microbiological Testing by the Dairy Industry

Samples taken for regulatory purposes must be analyzed for TBC, CC, and SCC according to approved methods that have been collaboratively tested (Wehr and Frank, 2004) and conducted in accordance with NCIMS laboratory procedures. While the TBC provides an estimate of the overall quality of raw milk for regulatory purposes, it may not provide sufficient information regarding the level of psychrotrophic bacteria, nor is it useful for predictive information on the shelf life of milk and milk products. In addition, the TBC does not provide an indication of pathogen levels. For example, a very low TBC is no assurance that raw milk is pathogen-free.

Many dairy cooperatives and dairy manufacturing facilities offer incentive or premium programs for bulk tank milk with lower TBC and SCC than the regulatory limit. For example, recommended achievable goals for raw milk premium programs are TBC < 10,000 CFU/mL and SCC < 350,000 cells/mL (Sawant and Campbell, 2007). Considerable research effort has been expended over the past 40 years to develop microbiological plating methods that will provide milk cooperatives and processors with additional information regarding the shelf life and keeping quality of milk and milk products. Some of these plating methods that are used in dairy quality control include:

- *Psychrotrophic bacteria count (PBC)*. This method is essentially an SPC whereby the plates are incubated at 7°C (45°F) for 10 days (Wehr and Frank, 2004). The test, which is usually done on pasteurized milk, has the obvious disadvantage of the time required for results.
- *Preliminary incubation count (PIC)*. This method is an improvement to the PBC in which the raw milk sample is pre-incubated at 12.7°C (55°F) for 18 hours, prior to determining a TBC (Wehr and Frank, 2004). Variations of the PIC are conducted on both raw and pasteurized milk samples. While the PIC is a slight improvement over PBC in

regard to time required, the time element is still a disadvantage. The PIC does not provide information as to the type of bacteria present, nor does it indicate the source of contamination. A recommended achievable goal for bulk tank milk premium program is PIC < 20,000 CFU/mL (Sawant and Campbell, 2007).

- *Gram-negative bacteria count (GNC)*. The intent of the GNC is to select for Gram-negative bacteria by using crystal violet tetrazolium (CVT) agar which inhibits the growth of Gram-positive bacteria and plating is done at 21°C (69.8°F; Wehr and Frank, 2004).
- *Thermotolerant count (TC) or laboratory pasteurized count (LPC)*. In this method, raw milk is heated at 62.8°C (145°F) for 30 minutes and cooled, then plated using a TBC method (Wehr and Frank, 2004). This method is intended to give an estimate of the thermotolerant and thermoresistant bacteria. Traditionally run on raw milk, the LPC was thought to give an indication of cleaning and sanitizing level on dairy farms. While many dairy quality laboratories abandoned this method in favor of the PIC, it may be finding renewed use in the future. An achievable goal under incentive programs may be a TC < 300 CFU/mL (Sawant and Campbell, 2007).
- *PI coliform count (PICC)*. In this method, milk is preincubated at 90°F (32°C) for 24 hours, prior to conducting the CC. Variations of this method have been termed *stressed coliform count*. If applied to raw milk, the PICC may provide additional information with regard to insanitary conditions on the farm, especially with regard to coliform mastitis. An achievable goal for bulk milk premium programs may be a PICC < 50 CFU/mL (Sawant and Campbell 2007). Since the PICC allows for recovery of injured cells, it may have an advantage over the CC for evaluating the quality of pasteurized milk products (e.g., cheese, ice cream).

Bigalke (1984) suggested that an ideal method to determine milk microbiological quality should include the following criteria:

- rapid,
- economical,
- reflect the total number of microorganisms,
- reflect the psychrotrophic microorganism,
- reflect the sanitation conditions, and
- reflect the storage conditions of the milk.

Table 5.9. Minimal Milk Pasteurization Requirements in the United States (PMO, 2005)

Pasteurization Process	Product Category	Temperature/Time
Vat or batch pasteurization	Milk	145°F (63°C)/30 minutes
	10% fat or higher	150°F (66°C)/30 minutes
	Added sweeteners	150°F (66°C)/30 minutes
	Concentrated (condensed)	150°F (66°C)/30 minutes
High temperature short time (HTST)	Milk	161°F (72°C)/15 seconds
	10% fat or higher	166°F (75°C)/15 seconds
	Added sweeteners	166°F (75°C)/15 seconds
	Concentrated (condensed)	166°F (75°C)/15 seconds
	Eggnog	175°F (80°C)/25 seconds
Ultra-pasteurization	Milk	280.4°F (138°C)/2 seconds
Ultrahigh temperature (UHT) aseptic	Milk	Comply with low acid canned food regulations (21 CFR 113)

Many rapid methods are being developed which measure metabolites or cellular components (Griffiths 2000). In addition, highly selective for specific microorganisms (e.g., PCR and related methods) are on the horizon. However, these rapid methods are not routinely used dairy quality laboratories. It is anticipated, however, that as the dairy industry moves to longer shelf-life products, that more specific rapid methods will become more accepted.

PROCESSING AND HANDLING INTERVENTIONS

In the foregoing, a variety of microorganisms were discussed. However, through strict regulatory compliance, and diligent industry response, the level of milk and milk product contamination has been minimized with improved safety and quality. In addition to strict adherence to pasteurization requirements, the dairy industry has adopted innovations in sanitary design of equipment and facilities, as well as improved testing methodology. In addition, the industry has weathered the storm of high impact pathogens with considerable improvement in regard to safety as well as quality of milk and milk products.

Milk Pasteurization

The first pasteurization requirements in the United States were directed at inactivation of tuberculosis causative organisms (e.g., *Myco. bovis*, *Myco. tuberculosis*). These pasteurization requirements were later increased to assure the inactivation of *Cox. burnetii*, the causative agent for Q fever (Holsinger et al., 1997; NACMF, 2003). Milk pasteurization is

defined as the process of heating every particle of milk or milk products in properly designed and operated equipment to a given temperature and holding at or above that temperature for at least the corresponding specific time (PMO, 2005). The pasteurization requirement for milk pasteurization of milk and milk products is shown in Table 5.9. Heat treatments (temperature and time) which are equivalent to the values shown are also listed in the PMO.

It is common industry practice to pasteurize milk and milk products at higher temperatures than the minimum required in the PMO. In addition, higher heat treatment is required for functional purposes in milk used for the manufacture of buttermilk, yogurt, and related cultured dairy products.

Processing Innovations

There are a number of technologies that are being investigated as alternatives to heat pasteurization (Griffiths 2000), or for use in conjunction with pasteurization, including high-pressure processing, ohmic and pulsed electric heating, microfiltration, bacterofugation, and ultrasound. In general, these methods may avoid heat-induced flavor changes associated with heat pasteurization. In addition, if used in combination with pasteurization, they may provide additional protection against heat-resistant spoilage microorganisms. For example, microfiltration and bacterofugation are being used commercially in combination with HTST pasteurization in Canada.

A wide range of processing, refrigeration, and sanitation technologies are involved in the manufacture of milk products with extended shelf life (Cromie, 1991). However, the term, *extended shelf life* (ESL)

is ill-defined and has differing definition throughout the world. U.S. regulations under the PMO define three categories (e.g., pasteurized milk, ultra-pasteurized milk, UHT milk) according to their heat treatments (Table 5.9). UHT milk is a sterile, shelf-stable product that does not require refrigeration, while ultra-pasteurized products require refrigeration. Since extending shelf life with traditional pasteurization and packaging beyond 20–25 days is difficult, ESL milk has traditionally referred to refrigerated milk products with shelf life between 25 and 45 days. However, aseptic and other forms of packaging innovations have extended this definition to 45–60 days. To differentiate between the milk product categories of extended shelf-life refrigerated milk, Henyon (1999) suggested that a major difference between ESL milk and aseptic milk is that ESL milk is not packaged aseptically in a sterile environment. It is anticipated that the definition of ESL milk and milk products will be debated further, and perhaps, will be more clearly defined in the future.

The use of additional food-grade antimicrobial treatments and agents to extend the shelf life of cottage cheese, and other cultured dairy products has the topic of considerable research investigation and many of these treatments and agents are in commercial use. The use of carbon dioxide as a processing adjunct in cottage cheese and other cultured products has had favorable response by the dairy industry and a variety of processing methods are being used commercially (Hotchiss et al., 2006; Mermelstein, 1997). In addition, a variety of antimicrobial agents (e.g., nisin, pimaricin, propionate, and sorbate) are under investigation or have commercial application for control of psychrotrophic spoilage bacteria and molds in cultured dairy products (Tortorello et al., 1991). In addition, mixtures of inhibitory bacteria (e.g., *Lac. lactis* subsp. *diacetylactis*, *Pr. shermanii*; Boudreaux et al., 1988) as well as other LAB preparations, are commercially available for use in extending the shelf life of cultured dairy products.

Dairy Sanitation Innovations

The dairy industry has historically been a leader with regard to adopting innovations in the materials, fabrication, surface finish and design of food contact surfaces and equipment (3-A Sanitary Standards, Inc., 2007; Frank and Chmielewski, 2001; Schmidt and Erickson, 2005a) as well as sanitary design of facilities (Schmidt and Erickson, 2005b). Food contact surface innovations, especially in rubber and plas-

tic materials, will undoubtedly bring additional questions with regard to cleanability as well as methodology to determine cleanability. More aggressive use of environmental sanitizing agents in the dairy industry, has created a need for more durable, impervious environmental (e.g., nonproduct contact) surfaces in the dairy plant.

There has been considerable interest in implementing the hazard analysis critical control point (HACCP) system in the dairy industry (see Chapter 22 for details). The FDA and the NCIMS have recently implemented voluntary HACCP system for Grade A fluid milk and milk product processing facilities.

Airborne contamination is an important vector of microbial contamination of milk and milk products. Aerosols may be associated with plant workers, floor drains, ventilation systems, supplies, milk and water pooling on floors and conveyor systems (Hedrick and Heldman, 1969). In recent years, the research knowledge of the mechanism and detection of airborne contamination has improved, but air sampling innovations have been slow to be adopted by dairy manufacturers in routine quality control (Ren and Frank, 1992).

The formation and properties of microbial biofilm on food contact surfaces in dairy plants has also been investigated. Bacterial biofilm form through attachment mechanisms of the specific bacteria, and the potential for biofilm formation is increased by the presence of food soil on the surface. Many food-related bacteria (e.g., *S. typhimurium*, *L. monocytogenes*, *Ps. fluorescens*, *Ps. fragi*, *E. coli* O157:H7), readily adhere and form biofilm to a varied degree on food contact surfaces (Hood and Zottola, 1997). NSLAB biofilms have been investigated in cheese manufacturing (Somers et al., 2001), and *L. curvatus* biofilm on cheese handling equipment may lead to calcium lactate crystal formation in Cheddar cheese (Wong, 1998). Multi-species biofilm may be formed whereby bacteria with well-defined extracellular polymeric substances (EPS) for attachment (e.g., *Pseudomonas* spp.) may precede the attachment of other microorganisms, such as *L. monocytogenes* (Jeong and Frank, 1994). Attached microorganisms are more resistant than freeliving microorganisms (Folsom and Frank, 2006; Hood and Zottola, 1995). In addition, biofilms are difficult to remove by routine cleaning and sanitizing procedures (Antoniou and Frank, 2005), and require oxidative cleaners and sanitizing chemicals for their removal (Folsom and Frank, 2006).

CURRENT AND FUTURE MICROBIOLOGICAL ISSUES

There are three microbiological issues that will be treated separately in this chapter. These include consumption of raw milk, the aging requirement for cheese manufactured from raw milk, and *Mycobacterium avium* subsp. *paratuberculosis* (MAP). These three controversial issues have received considerable internet and media attention in recent years.

Raw Milk Consumption

Federal regulation of milk pasteurization and sanitation in dairy processing plants has been in existence in the United States for nearly 100 years. Further, as a public health control procedure, the milk pasteurization process (or equivalent) has been recognized throughout the world. As a result of improved sanitation and pasteurization, the incidence of milk-borne illness in the United States has decreased from approximately 25% of all reported foodborne illness outbreaks in 1938 to less than 1% of reported outbreaks today. Similar trends have been observed internationally with mandatory milk pasteurization having a significant positive impact on public health and safety in many countries.

Since 1987, pasteurization is required for all packaged milk and milk products for human consumption in interstate commerce. In spite of this, there is a customer clientele, that appears to be increasing, that has a demand for raw milk for a variety of reasons. Therefore, the majority of state regulations currently allow raw milk sales with certain limitations and legislative changes have been or are being proposed in many other states to allow raw milk sales.

The apparent increase in raw milk enthusiasts may be directly related to efforts of highly effective organizations such as the Weston A. Price Foundation (WAPF, 2007) and the Natural Milk Coalition of Canada (NMCC, 2007) which have actively sought to overturn state regulations prohibiting the sale of raw milk. These organizations are enthusiastically promoting raw milk consumption by making unsubstantiated and false claims regarding the health benefit achieved by drinking raw milk, and have suggested that consumption of pasteurized milk may be harmful. At the cornerstone of these claims, are assumptions that:

- raw milk is teeming with helpful bacteria (LAB),
- raw milk has strong antimicrobial systems (e.g., lactoperoxidase, lactoferrin, and lysozyme),

- raw milk boosts the immune system, and
- pasteurization destroys the nutritional value as well as destroying these therapeutic and antimicrobial properties.

These claims have been refuted by many experts in dairy technology and public health (FDA, 2003). While the antimicrobial systems mentioned above are present in raw milk, they need to be activated and do not protect the consumer if the pathogen load is high. After perusal of the list of pathogens associated with raw milk presented in this chapter and the frequency and news media coverage of foodborne illness outbreaks related to raw milk in recent years, the obvious conclusion is that the risks of raw milk consumption far outweigh any perceived benefits.

Aging Requirements for Cheeses Manufactured from Raw Milk

Currently, U.S. standards of identity for cheese products (21 CFR 133.150), require pasteurization for certain natural cheeses. However, most aged cheese may be manufactured from either raw or pasteurized milk. However, to insure the safety of the product, cheese made from raw milk must be aged 60 days at 1.7°C (35°F) or less. However, it has been demonstrated via inoculation studies that *L. monocytogenes* (Ryser and Marth, 1987) and *E. coli* O157:H7 (Reitsma and Henning, 1996) survive in Cheddar cheese well beyond this 60 days holding period. FDA commenced a compliance program initiative (FDA, 1998), which involved increased regulatory surveillance of domestic and imported cheese made from unpasteurized milk, plus a 3-year research study to assess the survival of pathogens in hard cheese made from unpasteurized milk. The appropriateness of the 60 days aging requirement is still under scrutiny. In the National Academy of Science (NAS) report (Anonymous 2003a), the committee recommended the development and implementation of scientifically appropriate performance standards for the reduction of targeted pathogens in finished products as a consequence of processing strategies or aging periods, and recommended pasteurization for products for which adequate pathogen reduction may not occur during manufacturing. Further, the committee recommended that FDA require that cheeses made from subpasteurized milk be prominently labeled as such.

Mycobacterium avium* subsp. *paratuberculosis

MAP is the causative agent for Johne's disease, a transmissible disease estimated to affect approximately 22% of the U.S. dairy herds (USDA, 1997). Considerable effort has been expended at developing effective methods for preventing MAP on dairy farms including on-farm pasteurization of waste milk fed to calves (Stabel, 2001; Stabel et al., 2004).

While some reports suggest a relationship between MAP and Crohn's disease in humans (Chiodini and Rossiter, 1996; Sung and Collins, 1998), the significance of MAP as a human pathogen and potential causative agent for Crohn's disease is unknown and is still under investigation (Grant, 2005; Jones et al., 2006; NACMCF, 2007; Stabel, 2000).

The methodology for detection of MAP is cumbersome and the reliability of some of the methods used for isolation of this bacterium are not completely reliable with false-positives being possible, as well as potential cross-contamination issues in the laboratory (Mendez et al., 2006). Nonetheless, MAP has been isolated from raw milk as well as from pasteurized milk and milk products in several countries (Grant et al., 2002; Jayarao et al., 2005; O'Reilly et al., 2004).

The significance of MAP, and its relationship to food products, has been recently evaluated in a comprehensive National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2007) report. According to this review:

- the percentage of raw milk samples testing positive ranged from 7.8 to 27.5% (for those investigations using PCR technique), compared to 0.25–11.1% (for those investigations using culture analyses); and
- the percentage of pasteurized milk testing positive ranged from 2 to 15% (for those investigations using PCR), compared to 0–2.8% (for studies using culture analyses).

If present in pasteurized milk and milk products, MAP may have survived the heat treatment conditions used in the pasteurization process, but may also have entered the product through to postpasteurization contamination. The heat inactivation of MAP by milk pasteurization has been questioned, and there is variation in literature reports (Grant et al., 2002, 2005; Lund et al., 2002; Stabel and Lambertz, 2004; Sung and Collins, 1998). This variation is related to several factors (NACMCF, 2007) including laboratory inoculation versus natural contamination,

method of heat inactivation (capillary tube technique vs. pilot or commercial pasteurizing equipment), degree of turbulent flow, degree of cellular clumping, presence or absence of resuscitation step after heating, and volume of sample used.

While more research is needed, it appears that commercial pasteurization in properly operating equipment is adequate to inactivate MAP and that the risk of exposure to MAP in pasteurized dairy products is low. Some of the conclusions of the NACMCF report are as follows:

- "A standard detection method needs to be developed and adopted in order to accurately determine presence or absence of MAP in foods and other sources";
- "Milk, particularly raw milk, may be a likely food source of human exposure to MAP";
- "Thermal processes that deliver a 4–7 log reduction in the number of MAP cells should be adequate to inactivate the numbers of MAP estimated to be present in raw milk";
- "A small percentage (<3%) of commercially pasteurized milk may contain small numbers of viable MAP cells";
- Cheese made from pasteurized milk is unlikely to be a significant source of exposure to MAP, but the potential for exposure to MAP from milk products made from raw milk is unknown."

Other Microbiological Issues

It may be difficult to predict what microbiological issue may be lurking in the future. The U.S. dairy industry and its regulation are becoming more global with increased importation, as well as exportation, of dairy products. This will lead to more harmonization of dairy regulations. As the United States becomes more involved in exporting refrigerated products, there may be additional pressures to increase shelf life, and there may be additional microbiological concerns which will impact the processing and packaging methods used. The dairy industry has had a good track record compared to other food industry segments in recent years with regard to minimizing pathogens (especially *L. monocytogenes*) in the plant environments. However, the diligence must continue.

In recent years, the food industry has been characterized by new product innovations. Those that are related to the dairy sector include functional and/or probiotic dairy-based foods, and blended products of milk, with fruit juices. As many of the functional foods are targeted to the immunocompromised

populations (e.g., use of probiotic bacteria to boost immune systems, colostrum milk-based products), there will be additional customer demands with regard to processing and microbiological considerations. With regard to the blended milk and juice products, many of these products being produced in facilities that have not traditionally handled milk, and by processors may not have the knowledge of the sensitivity of milk and milk ingredients. If the manufacture is being done in facilities and equipment with inadequate sanitary design and using pasteurization equipment that may not have adequate safeguards, this could lead to microbiological concerns in the future.

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6

Regulations for Product Standards and Labeling

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U.S. Code of Federal Regulations
U.S. Product Standards of Identity
Fermented Milk and Milk Products
Cheese and Related Products
Ice Cream and Frozen Desserts
Food Additives and Packaging
Labeling

Glossary
Bibliography

U.S. CODE OF FEDERAL REGULATIONS

The U.S. Code of Federal Regulations (CFR) published by the U.S. government is a set of comprehensive documents containing all Federal Regulations. Each branch of government is assigned a different numerical title. The regulations for Food and Drugs are published in Title 21. These publications are revised and issued yearly. The current version can also be found online. The Title 21 CFR Parts 1–199 lays out U.S. Food and Drug Administration's (FDA) regulations for current good manufacturing practices, food labeling, standards of identity, and approved food additives.

U.S. PRODUCT STANDARDS OF IDENTITY

Food standards were established to promote fair competition among manufacturers and to eliminate consumer confusion. Currently, there are 97 federal standards of identity for various dairy products out of a total of 262 standards for all foods including dairy. Many states have also promulgated standards of identity for dairy products. The Nutrition Labeling Education Act (NLEA) of 1990 established that where federal and state standards exist simultaneously, the

federal standard preempts the state regulation. However, in the event no federal standard exists for a specific dairy product and a state standard has been promulgated, the state standard is in effect. In terms of detailed presentation, this manual addresses only federal standards of identity. For the most part, standards of identity dictate the processing procedure, composition and allowed ingredients of the product, and often cover public safety concerns and product labeling. All federal standards of identity for dairy products are referenced in Title 21 CFR, Parts 130–135. The Grade “A” Pasteurized Milk Ordinance (PMO), a model regulation for milk sanitation, adopts by reference the federal standards of identity. These standards of identity apply to products that are manufactured for sale in the United States including both domestically produced and imported products. The milk and cream standards are found in 21 CFR Part 131 which include definition of milk, cream, and dairy ingredients.

Milk

Description of Process

- Complete milking of healthy cows properly fed and housed
- Free from colostrum
- For beverage, milk has to be pasteurized or ultra-pasteurized
- Composition can be achieved by completely separating fat from milk and then adding back required amount of fat to desired level
- Other methods of standardization include addition of cream, concentrated milk, dry whole milk, skim milk, concentrated skim milk, or nonfat dry milk
- Milk may be homogenized

Composition

- Minimum of 8.25% milk solids-not-fat (MSNF)
- Minimum of 3.25% milk fat

Other Ingredients

- Vitamins A and D (minimum 2,000 IU vitamin A and total vitamin D content to be 400 IU)
- Carriers for vitamins A and D
- Characterizing flavoring ingredients (with or without coloring, nutritive sweetener, emulsifiers and stabilizers)

Nomenclature. The name of the food is “milk.” The addition of vitamins and characterizing flavorings must be included with the term “milk.”

Concentrated Milk**Process**

- Obtained by partial removal of water from milk
- Generally, this is done under vacuum
- Another name for such a product is condensed milk
- Can be pasteurized and homogenized

Composition

- Minimum total solids 25.5%
- Minimum milk fat content 7.5% the milk fat and total milk solids

Other Ingredients

- Vitamins A and D may be added
- Carriers for vitamins are allowed
- Characterizing flavors may be added

Nomenclature. Concentrated milk when starting with milk. Concentrated skim milk when starting with skim milk.

Sweetened Condensed Milk**Process**

- Partial removal of water from a mixture of milk and nutritive sweetener
- May be pasteurized and homogenized

Composition

- Minimum 8% milk fat

- Minimum 28% total milk solids
- Enough sweetener to prevent spoilage

Other Ingredients

- Flavoring ingredients
- Approved coloring agents
- Natural and artificial flavors

Nomenclature. The name of the food shall be sweetened condensed milk.

Evaporated Milk**Process**

- Obtained by partial removal of water from milk
- Product in container has to be thermally processed to achieve commercial sterility
- May be pasteurized and homogenized

Composition

- Minimum 6.5% milk fat
- Minimum 16.5% MSNF
- Minimum 23% total milk solids

Other Ingredients

- May add vitamins A and D
- Emulsifier
- Stabilizers
- Characterizing flavoring materials

Nomenclature. The name of the food shall be evaporated milk.

Dry Whole Milk**Process**

- Removal of most of the water from pasteurized milk
- Milk may be homogenized
- May also be blending milk, condensed milk, or dried nonfat with liquid or dried cream

Composition

- Minimum 26% and maximum 40% milk fat
- Maximum 5% moisture based on nonfat milk solids

Other Ingredients

- Vitamins A and D
- Carriers for vitamins A and D
- Emulsifier
- Stabilizers
- Anticaking agents
- Antioxidants
- Characterizing flavoring ingredients (with or without coloring and nutritive carbohydrate sweetener)

Nomenclature. The name of the food shall be dry whole milk.

Nonfat Dry Milk

Process. Product obtained by removal of water only from pasteurized skim milk.

Composition

- Maximum 5%
- Maximum 1.5% milk fat

Other Ingredients

- Vitamins A and D, when vitamins are added, the mixture has to meet standards for nonfat dry milk fortifier with vitamins A and D (21 CFR 131.127)
- Carriers for vitamins A and D
- Characterizing flavoring ingredients (with or without coloring and nutritive carbohydrate sweetener)

Nomenclature. The name of the food shall be nonfat dry milk. When vitamins are added the food shall be named nonfat dry milk fortifier with vitamins A and D.

Cream**Process**

- Cream means the liquid milk product high in fat separated from milk
- Fat content can be standardized by adding milk, concentrated milk, dry whole milk, skim milk, concentrated skim milk, or nonfat dry milk
- Cream must be heat-treated to be called pasteurized
- Time-temperature combinations recommended are 145°F for 30 minutes or 161°F for 15 seconds,

or 191°F for 1 second or 204°F for 0.05 second, or 212°F for 0.01 second

Composition

- Minimum 18% milk fat

Half-and-Half**Process**

- Mixture of milk and cream
- Pasteurized and homogenized
- May be ultra-pasteurized

Composition

- Minimum 10.5% milk fat
- Maximum 17.9% milk fat

Other Ingredients

- Emulsifier
- Stabilizers
- Nutritive sweeteners
- Characterizing flavoring ingredients (with or without coloring)

Nomenclature. The name of the food shall be half-and-half.

Heavy Cream**Process**

- Separation of fat from milk
- Standardization of fat content by various means
- Pasteurized or ultra-pasteurized
- May be homogenized

Composition

- Minimum 36% milk fat

Other Ingredients

- Emulsifier
- Stabilizers
- Nutritive sweeteners
- Characterizing flavoring ingredients (with or without coloring)

Nomenclature. The name of the food shall be heavy cream.

Light Cream

Process

- Partial removal of skim milk from cream
- Pasteurized or ultra-pasteurized
- May be homogenized

Composition

- Minimum 18% milk fat
- Maximum 29.9% milk fat

Other Ingredients

- Stabilizers
- Emulsifier
- Nutritive sweeteners
- Characterizing flavoring ingredients (with or without coloring)

Nomenclature. The name of the food shall be light cream, table cream, or coffee cream.

Light Whipping Cream

Process

- Concentrating milk fat from milk
- Pasteurized or ultra-pasteurized
- May be homogenized

Composition

- Minimum 30% milk fat
- Maximum 36% milk fat

Other Ingredients

- Emulsifier
- Stabilizers
- Nutritive sweeteners
- Characterizing flavoring ingredients (with or without coloring)

Nomenclature. The name of the food shall be whipping cream or light whipping cream.

Dry Cream

Process

- Removal of water only from pasteurized milk or a mixture of cream and milk
- It may also be manufactured by blending ingredients to achieve the required composition

Composition

- Minimum 40% milk fat
- Maximum 74.9% milk fat
- Maximum 5% moisture on MSNF basis

Other Ingredients

- Emulsifier
- Stabilizers
- Anticaking agents
- Antioxidants
- Nutritive carbohydrate sweetener
- Characterizing flavoring ingredients (with or without coloring)

Nomenclature. The name of the food shall be dry cream.

Whipped Cream

Composition

- Made by whipping the cream

Composition. Same as cream 21 CFR 131.150.

Other Ingredients

- Flavorings
- Sweeteners

Nomenclature. The unqualified name “whipped cream” should not be applied to any product other than one made by whipping the cream that complies with the standards of identity for whipping cream (Sections 131.150 and 131.157).

FERMENTED MILK AND MILK PRODUCTS

Specific requirements for fermented milk products such as cultured milk, sour cream, and yogurts are listed below.

Cultured Milk

Process

- Prepared by culturing with characterizing microbial organisms with one or more of the following: cream, milk, partially skimmed milk, and skim milk

- Must be pasteurized or ultra-pasteurized prior to the addition of the microbial culture and may be homogenized
- May contain other ingredients listed below

Composition

- Minimum of 3.25% of milk fat
- Minimum of 8.25% MSNF
- Minimum of 0.5% titratable acidity (expressed as lactic acid)
- 2,000 IU of vitamin A/qt (optional)
- 400 IU of vitamin D/qt (optional)

Other Ingredients

- Acidifying ingredients such as acetic acid, adipic acid, citric acid, fumaric acid, glucono-*delta*-lactone, hydrochloric acid, lactic acid, malic acid, phosphoric acid, succinic acid, or tartaric acid.
- Optional dairy ingredients include concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins, and whey (modified by partial or complete removal of lactose and/or minerals).
- The provision in the standard of identity for cultured milk limiting the sources of optional dairy ingredients has been stayed, pending the outcome of a public hearing. Other milk-derived ingredients (e.g., caseinates) may be used to increase the nonfat solids content in cultured milk.
- Nutritive carbohydrate sweeteners such as beet or cane sugar (sucrose), inverted sugar (paste or syrup), brown sugar, refined sugar, molasses (not blackstrap), high-fructose corn syrup, fructose, fructose syrup, maltose, maltose syrup, dried maltose syrup, malt extract, dried malt extract, honey, maple sugar, dextrose anhydrous, dextrose monohydrate, glucose syrup, dried glucose syrup, lactose, cane syrup, maple syrup, and sorghum.
- Flavoring.
- Color additives may be added, except those that impart butterfat or milk fat, color may not be added directly to the fluid product so that it gives the appearance that the product contains more milk fat than it actually does.
- Stabilizers.
- Butterfat or milk fat in the form of granules or flakes (which may or may not contain color additives).
- Aroma and flavor producing microbial culture.
- Salt.

- Flavor precursors (citric acid 0.15% maximum of milk or equal the amount of sodium citrate).

Nomenclature. The name of the food is “cultured milk.”

Milk fat level

- Milk fat percentage declaration is not required.

Process

- If the dairy ingredients were homogenized, then the label may indicate “homogenized” (optional).

Sweetened

- If sweetened with a nutritive carbohydrate sweetener without a characterizing flavor, then the label must indicate “sweetened.”

Characterizing organisms

- Name of the food may declare traditional or generic name of characterizing microbial organisms (optional) or ingredients, for example, “kefi -cultured milk,” “acidophilus-cultured milk,” or when lactic acid producing organisms are used “cultured buttermilk.”

Flavoring

- If characterizing flavors were added, then the name should indicate the common or usual name of the flavoring.

Sour Cream (Cultured Sour Cream)

Process

- Produced from souring pasteurized cream with lactic acid producing bacteria.
- May contain other ingredients listed below.

Composition

- Minimum of 18% milk fat
- Minimum of 14.4% milk fat for bulky flavored sour creams
- Minimum of 0.5% titratable acidity

Other Ingredients

- Ingredients which improve texture, prevent syneresis, or extend shelf life of the sour cream

- Flavor precursor—sodium citrate in a minimum quantity of 0.1% added prior to culturing
- Rennet
- Salt
- Flavoring ingredients with or without coloring, fruit or fruit juice (may be from concentrate), or natural and artificial flavoring

Nomenclature. The name of the food is “sour cream” or “cultured sour cream.”

Flavoring.

- If characterizing flavors were added, then the name should indicate the common or usual name of the flavoring.

Sweetened. If the sour cream was sweetened with a nutritive sweetener without the addition of characterizing flavorings, then the label must indicate “sweetened.”

Yogurt (Includes Drinkable Yogurts)

Process

- Produced by culturing with the lactic acid-producing bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (may contain other lactic acid-producing bacteria) one or more of the following: cream, milk, partially skimmed milk, skim milk, or reconstituted dairy ingredients. The standard of identity for yogurt does not include the addition of reconstituted dairy ingredients as basic components in the manufacturing of yogurt. This exclusion has been stayed, pending the outcome of a public hearing, and therefore, reconstituted dairy ingredients could be used as a basic dairy component in yogurt.
- May be homogenized and must be pasteurized or ultra-pasteurized prior to the addition of bacteria culture.
- Flavoring ingredients may be added after pasteurization or ultra-pasteurization.
- The product may be heat-treated to destroy viable microorganisms to extend shelf life.
- May contain other ingredients listed.

Composition. The provision requiring the milk fat level to be a minimum of 3.25% milk fat before the addition of bulky flavorings has been stayed, pending the outcome of a public hearing.

- Minimum of 8.25% MSNF
- 2,000 IU vitamin A/qt (optional)
- 400 IU vitamin D/qt (optional)
- The product does not have to meet the titratable acidity requirement indicated in the standard (minimum of 0.9% titratable acidity). This provision was stayed, pending the outcome of a public hearing.

Other Ingredients

- Optional dairy ingredients include concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins, and whey (modified by partial or complete removal of lactose and/or minerals). The provision in the standard of identity for yogurt limiting the sources of optional dairy ingredients has been stayed, pending the outcome of a public hearing. Other milk-derived ingredients (e.g., caseinates) may be used to increase the nonfat solids content in yogurt.
- Nutritive carbohydrate sweeteners such as beet or cane sugar (sucrose), inverted sugar (paste or syrup), brown sugar, refined sugar, molasses (not blackstrap), high-fructose corn syrup, fructose, fructose syrup, maltose, maltose syrup, dried maltose syrup, malt extract, dried malt extract, honey, maple sugar, dextrose anhydrous, dextrose monohydrate, glucose syrup, dried glucose syrup, lactose, cane syrup, maple syrup, and sorghum.
- Flavoring ingredients.
- Color additives.
- Stabilizers.
- Preservatives as functional ingredients were not provided for in the standard of identity for yogurt. This exclusion has been stayed, pending the outcome of a public hearing, and therefore, preservatives could be added to yogurt as a functional ingredient.

Nomenclature. The name of the food is “yogurt.” Alternate spelling of the food should not serve as the *name of the food* (e.g., “yogourt,” “yoghurt”).

Process

- If the dairy ingredients were heat-treated after culturing, then the name of the food must be followed by the parenthetical phrase (“heat-treated after culturing”).
- If the dairy ingredients were homogenized, then the label may indicate “homogenized” (optional).

Vitamins

- If vitamins are added, then the following phrase is stated as appropriate: “vitamin A” or “vitamin A added,” “vitamin D” or “vitamin D added,” or “vitamins A and D” or “vitamins A and D added.”
- The word “vitamin” may be abbreviated as “vit.”

Flavorings. If the yogurt contains characterizing flavorings, then the common or usual name of the flavorings shall be indicated in the name.

Sweetened. If the product is sweetened with a nutritive sweetener without any characterizing ingredients added, then the label must indicate “sweetened.”

Low-Fat Yogurt (Includes Drinkable Low-Fat Yogurts)

Same as yogurt except composition and nomenclature.

Composition. Either $\frac{1}{2}$, 1, $1\frac{1}{2}$, or 2% milk fat (before the addition of bulky flavorings).

Nomenclature. The name of the food is “low-fat yogurt.” Alternate spelling of the food should not serve as the *name of the food* (e.g., “low-fat yogourt,” “low-fat yoghurt”).

Milk fat level. The percentage of milk fat must be declared (not in decimal notation) as “ $\frac{1}{2}$ % milk fat,” “1% milk fat,” “ $1\frac{1}{2}$ % milk fat,” or “2% milk fat.”

Nonfat Yogurt (Includes Drinkable Nonfat Yogurts)

Same as yogurt except composition and nomenclature.

Composition

- Less than 0.5% milk fat (before the addition of bulky flavorings).

Nomenclature. The name of the food is “nonfat yogurt.” Alternate spelling of the food should not serve as the *name of the food* (e.g., “nonfat yogourt,” “nonfat yoghurt”).

Stayed Provisions

It should be noted that as part of FDA’s administrative procedures for enacting and updating standard, any person who would be adversely affected by a change in a food standard may file objections specifying the provisions being objected to, providing the grounds and requesting a public evidentiary hearing. The mere filing of the objection prevents the action from being taken (the action is stayed) and the FDA must hold a hearing.

Some requirements listed in the CFR have been stayed following the outcome of a public hearing. At the time of printing, FDA had not acted to proceed with such a public hearing. Therefore, the following provisions noted as being stayed are not in effect:

1. There is no restriction to those so named for the type of milk-derived ingredients that may be used to increase the nonfat solids content of cultured and acidified milks, eggnog, and yogurts.
2. Reconstituted dairy ingredients can be used as the basic ingredient in the manufacture of yogurts.
3. Preservatives can be added to yogurts.
4. There is no set minimum titratable acidity of 0.9%, expressed as lactic acid.
5. The requirement that the 3.25% minimum milk fat level is eliminated after the addition of one or more of the optional sources of MSNF for yogurt.

Proposed Changes to U.S. Standards for Yogurt and Fermented Milks

A citizen’s petition was filed in 2000 with FDA by the National Yogurt Association (NYA) on behalf of its members, requesting that FDA modernize the standards of identity for yogurt to replace the existing yogurt standards and make conforming amendments to the existing cultured milk standard of identity. As required under FDA’s procedural regulations, a citizen’s petition must include information regarding the action requested, statement of grounds, environmental impact, economic impact, and certification of all relevant information, both favorable and unfavorable. The regulations also require FDA to rule on each petition filed with the Agency.

NYA’s petition provides the basis for the FDA to consider changes that would replace the currently existing fragmented standards for yogurt, low-fat yogurt, and nonfat yogurt as these standards contain numerous stayed provisions. The proposed standards would require that yogurt contain a minimum level of certain live and active bacterial cultures and allow

for more flexibility to implement advances in food technology.

The specific details of the proposed changes are as follows:

- *Single Standard of Identity*: Incorporates full-fat, low-fat, nonfat standards in one standard of identity. It also suggests that a parallel “cultured/fermented milk” standard be created for similar products that do not meet the new yogurt standard.
- *Live and Active Characterizing Cultures*: Require that yogurt be characterized by certain levels of bacterial cultures of at least 10^7 CFU/g active cultures *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus* at the time of manufacture.
- *Acidity*: Originally, the petition proposed a minimum acidity for yogurt of pH 4.6 or lower rather than a titratable acidity. Later, this request was modified to maintain titratable acidity as the measure of lactic acid production and recommend a standard of 0.6% titratable acidity, which more closely reflects industry practice and consumer preference for less tart yogurt than the present 0.9% lactic acid.
- *Homogenization/Pasteurization*: Clarifies that the standard dairy ingredients must be pasteurized or ultra-pasteurized before culturing and that other ingredients may be added after pasteurization and culturing.
- *Standard Dairy Ingredients*: Permits the use of reconstituted dairy ingredients as the basic dairy ingredients used to compose the minimum 8.25% nonfat milk solids. Restricts whey protein concentrate to be used as a dairy ingredient in levels up to 25% of all nonfat milk solids.
- *Other ingredients*: Permits any “milk-derived ingredients used for technical or functional purpose.” Requires that dairy ingredients comprise at least 51% of the product’s overall ingredients by weight. Clarifies that other bacterial cultures, in addition to the two characterizing cultures, are permitted. Also allows any safe and suitable nutritive carbohydrate sweeteners or nonnutritive sweeteners, flavoring ingredients, color additives, stabilizers and emulsifiers, preservatives, vitamins, and minerals, and safe and suitable ingredients added for nutritional or functional purposes.
- *Nomenclature*: Characterizes products containing more than 3.0 g of total fat per reference amount

commonly consumed (RACC) as “yogurt.”

Products with at least 0.5 g, but not more than 3.0 g of total fat per RACC will be named as “low-fat yogurt” and if the food is less than 0.5 g of total fat per RACC, it will be “nonfat yogurt.”

This change bases the identity of the product on the total fat quantity in the entire product rather than just the milk fat of the yogurt prior to addition of other ingredients or flavorings.

At the time of printing, FDA has not yet changed the standards of identity to incorporate these suggested modifications. Under the rule-making process, FDA must consider public comment for interested stakeholders before promulgating new standards of identity. The first step in this process occurred in early 2004 when FDA published an Advance Notice of Proposed Rule Making seeking comments on the proposed NYA petition. The next step is for FDA to consider the relevant comments and publish either a Proposed Rule allowing for additional comments or Final Rule Making. However, under FDA Procedures the law requires a very burdensome process for issuance, amendment, or repeal of standards of identity if anyone objects to the proposal being considered. Since some interested parties have filed support of the yogurt modernization petition and others have objected to specific provisions, it is not known when proposed changes might be finalized.

CHEESE AND RELATED PRODUCTS

Definition for milk, skim milk, cream, and pasteurized for this category of standardized foods are same as those given above. There are a variety of cheeses and related products and these are described below in alphabetical order. The general process for cheese manufacture is to add safe and suitable lactic acid bacteria to pasteurized milk or cream followed by the addition of approved milk-clotting enzymes derived from animal or microbial sources which leads to curd formation. The curd is cut and heated to separate the whey. The whey is drained and the curds are transferred to molds. Pressing of the molded curds is followed with certain varieties of cheeses and salt addition may take place prior to transfer of curds to a mold or alternatively after the molded and pressed curd is demolded. The specific procedures for cheese manufacture by variety are discussed in a following section.

Asiago Cheese

Composition

- Minimum 50% milk fat
- Maximum 45% moisture
- Cured for a minimum of 60 days

Optional Ingredients

- Color to neutralize yellowness
- Calcium chloride 0.02% by weight of milk
- Bleaching agents to include benzoyl peroxide (maximum 0.002% by weight of milk), potassium alum, calcium sulfate, and magnesium carbonate at levels not exceeding six times the amount of benzoyl peroxide
- Safe and suitable antimycotic agents on the surface of the cheese

Nomenclature. Asiago fresh cheese, Asiago soft cheese, is the food prepared from milk and other ingredients as specified

Asiago Medium Cheese

Same as Asiago fresh cheese except that the cheese has to be cured for a minimum of 6 months and contains a maximum of 35% moisture and a minimum of 45% milk fat.

Asiago Old Cheese

Same as Asiago fresh except that the cheese has to be aged a minimum of 1 year and contains a minimum of 42% milk fat and a maximum of 32% moisture.

Blue Cheese

Composition

- Bluish-green mold, *Penicillium roquefortii*, throughout the cheese
Minimum milk fat content is 50% by weight of the solids
Maximum moisture content is 46% by weight
- Blue cheese is at least 60 days old

Brick Cheese

The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 44% by weight. The cheese is cured at a temperature of not less than 35°F for at least 60 days.

Caciocavallo Siciliano Cheese

Process

- Use the milk of cow, goat, or sheep or a mixture of these
- Stringy texture
- Made in oblong shapes
- Cured for not less than 90 days at a temperature of not less than 35°F

Composition

- Contains not more than 40% of moisture
- Solids contain not less than 42% milk fat

Cheddar

- The minimum milk fat content is 50% by weight of the solids
- The maximum moisture content is 39% by weight
- If the dairy ingredients used are not pasteurized, the cheese is cured at a temperature of not less than 35°F for at least 60 days

Low-Sodium Cheddar Cheese

Compositional requirements are same as for Cheddar cheese. However, this type of cheese should contain not more than 96 mg of sodium per pound of finished food. The name of the food is “low-sodium cheddar cheese.” The letters in the words “low sodium” shall be of the same size and style of type as the letters in the words “cheddar cheese,” wherever such words appear on the label. If a salt substitute is used, the label shall bear the statement “___ added as a salt substitute,” the blank being filled in with the common name or names of the ingredient or ingredients used as a salt substitute.

Colby Cheese

It contains not more than 40% of moisture, and its solids contain not less than 50% of milk fat. If the milk used is not pasteurized, the cheese so made is cured at a temperature of not less than 35°F for not less than 60 days.

Low-Sodium Colby Cheese

Compositional requirements are same as that for Colby cheese except the finished product will contain not more than 96 mg/lb. The size of letters “low

sodium” will be the same as that for the low-sodium cheddar cheese and if salt substitutes are used, they should be mentioned on the label.

Cold-Pack and Club Cheese

Cold-pack cheese, club cheese, is the food prepared by comminuting, without the aid of heat, one or more cheeses of the same or two or more varieties, except cream cheese, Neufchatel cheese, cottage cheese, low-fat cottage cheese, cottage cheese dry curd, hard-grating cheese, semisoft part-skim cheese, part-skim spiced cheese, and skim milk cheese for manufacturing, into a homogeneous plastic mass.

All cheeses used in a cold-pack cheese are made from pasteurized milk or are held for not less than 60 days at a temperature of not less than 35°F before being comminuted.

Cold-Pack Cheese Food

Cold-pack cheese food is the food prepared by comminuting and mixing, without the aid of heat, one or more of the optional cheese ingredients may be used. All cheeses used in a cold-pack cheese food are made from pasteurized milk, or are held for not less than 60 days at a temperature of not less than 35°F before being comminuted. The moisture content of a cold-pack cheese food is not more than 44%, and the fat content is not less than 23%.

The weight of the cheese ingredient constitutes not less than 51% of the weight of the finished cold-pack cheese food.

Cold-Pack Cheese Food with Fruits Vegetables and Meat

Its milk fat content is not less than 22%. It contains one or any mixture of two or more of the following: any properly prepared fresh, cooked, canned, or dried vegetable; any properly prepared cooked or canned meat.

Cook Cheese or Koch cheese

The curd is cured for 2 or 3 days. It is then heated to a temperature of not less than 180°F until the hot curd will drop from a ladle with a consistency like that of honey. The hot cheese is filled into packages and cooled. The maximum moisture content is 80% by weight.

Cottage Cheese

Cottage cheese is the soft uncured cheese prepared by mixing cottage cheese dry curd with a creaming mixture. The milk fat content is not less than 4% by weight of the finished food, within limits of good manufacturing practice. The finished food contains not more than 80% of moisture. The creaming mixture is prepared from safe and suitable ingredients.

When the product is made using processes that are considered alternative, for example, direct acidification the name of the procedure has to appear prominently on the label. In this example, it should say “direct set” or directly acidified cottage cheese.

Cream Cheese

Cream cheese is the soft, uncured cheese. The minimum milk fat content is 33% by weight of the finished food, and the maximum moisture content is 55% by weight. Stabilizers may be used up to a maximum of 0.5%. Dioctyl sodium succinate may be used at a maximum level of 0.5% of the amount of stabilizer used. Other optional ingredients are also permitted.

Cream Cheese with Other Foods

Cream cheese with other foods is the class of foods prepared by mixing, with or without the aid of heat, cream cheese with one or a mixture of two or more types of foods (except other cheeses), in an amount sufficient to differentiate the mixture from cream cheese. The maximum moisture content of the mixture is 60% by weight. The minimum milk fat is 33% by weight of the cream cheese and in no case is less than 27% of the finished food. Other foods that may be added are properly prepared fresh, cooked, canned, or dried fruits or vegetables; cooked or canned meats, relishes, pickles, or other suitable foods.

Edam Cheese

Edam cheese must have a minimum milk fat content is 40% by weight of the solids and the maximum moisture content is 45% by weight. If the dairy ingredients used are not pasteurized, the cheese is cured at a temperature of not less than 35°F for at least 60 days.

Gammelost cheese

Gammelost cheese is the food prepared from non-fat milk; the maximum moisture content is 52% by weight.

Gorgonzola Cheese

It is characterized by the presence of bluish-green mold, *Penicillium roquefortii*, throughout the cheese. The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 42% by weight.

Gouda Cheese

Gouda cheese conforms to the definition and standard of identity and complies with the requirements for label declaration of ingredients prescribed for Edam cheese except that the minimum milk fat content is 46% by weight of the solids, and the maximum moisture content is 45% by weight.

Gruyere Cheese

Gruyere cheese contains small holes or eyes. It has a mild flavor, due in part to the growth of surface-curing agents. The minimum milk fat content is 45% by weight of the solids and the maximum moisture content is 39% by weight. The dairy ingredients used may be pasteurized. The cheese is at least 90 days old.

Limburger Cheese

The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 50% by weight. If the dairy ingredients used are not pasteurized, the cheese is cured at a temperature of not less than 35°F for at least 60 days.

Monterrey Cheese and Monterrey Jack Cheese

The minimum milk fat content is 50% by weight of the solids, and the maximum moisture content is 44% by weight. The dairy ingredients used are pasteurized.

Mozzarella Cheese and Scamorza Cheese

It may be molded into various shapes. The minimum milk fat content is 45% by weight of the solids, and the moisture content is more than 52% but not more

than 60% by weight. The dairy ingredients used are pasteurized.

Muenster and Munster Cheese

The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 46% by weight. The dairy ingredients used are pasteurized.

Neufchatel Cheese

Neufchatel cheese is a soft uncured cheese. The milk fat content is not less than 20% but less than 33% by weight of the finished food and the maximum moisture content is 65% by weight. The dairy ingredients used are pasteurized.

Parmesan and Reggiano Cheese

It is characterized by a granular texture and a hard and brittle rind. It grates readily. It contains not more than 32% of moisture, and its solids contain not less than 32% of milk fat. It is cured for not less than 10 months.

Pasteurized Process Cheese

Pasteurized process cheese is the food prepared by comminuting and mixing, with the aid of heat, one or more cheeses of the same or two or more varieties, except cream cheese, Neufchatel cheese, cottage cheese, low-fat cottage cheese, cottage cheese dry curd, cook cheese, hard-grating cheese, semisoft part-skim cheese, part-skim spiced cheese, and skim milk cheese for manufacturing with an emulsifying agent into a homogeneous plastic mass.

The moisture content of a pasteurized process cheese made from a single variety of cheese is not more than 1% greater than the maximum moisture content prescribed by the definition and standard of identity, if any there be, for the variety of cheese used; but in no case is more than 43%, except that the moisture content of pasteurized process washed curd cheese or pasteurized process Colby cheese is not more than 40%; the moisture content of pasteurized process Swiss cheese or pasteurized process gruyere cheese is not more than 44%; and the moisture content of pasteurized process limburger cheese is not more than 51%.

The fat content of the solids of a pasteurized process cheese made from a single variety of cheese is

not less than the minimum prescribed by the definition and standard of identity, if any there be, for the variety of cheese used, but in no case is less than 47%; except that the fat content of the solids of pasteurized process Swiss cheese is not less than 43%, and the fat content of the solids of pasteurized process gruyere cheese is not less than 45%.

The moisture content of a pasteurized process cheese made from two or more varieties of cheese is not more than 1% greater than the arithmetical average of the maximum moisture contents prescribed by the definition and standards of identity, if any there be, for the varieties of cheese used; but in no case is the moisture content more than 43%, except that the moisture content of a pasteurized process cheese made from two or more of the varieties cheddar cheese, washed curd cheese, Colby cheese, and granular cheese is not more than 40%, and the moisture content of a mixture of Swiss cheese and gruyere cheese is not more than 44%.

The fat content of the solids of a pasteurized process cheese made from two or more varieties of cheese is not less than the arithmetical average of the minimum fat contents prescribed by the definitions and standards of identity, if any there be, for the varieties of cheese used, but in no case is less than 47%, except that the fat content of the solids of a pasteurized process gruyere cheese made from a mixture of Swiss cheese and gruyere cheese is not less than 45%.

The weight of each variety of cheese in a pasteurized process cheese made from two varieties of cheese is not less than 25% of the total weight of both, except that the weight of blue cheese, nuworld cheese, Roquefort cheese, or gorgonzola cheese is not less than 10% of the total weight of both, and the weight of limburger cheese is not less than 5% of the total weight of both. The weight of each variety of cheese in a pasteurized process cheese made from three or more varieties of cheese is not less than 15% of the total weight of all, except that the weight of blue cheese, nuworld cheese, Roquefort cheese, or gorgonzola cheese is not less than 5% of the total weight of all, and the weight of limburger cheese is not less than 3% of the total weight of all. These limits do not apply to the quantity of cheddar cheese, washed curd cheese, Colby cheese, and granular cheese in mixtures that are designated as "American cheese."

Pasteurized Process Cheese Food

A pasteurized process cheese food is the food prepared by comminuting and mixing, with the aid of

heat, one or more of the optional cheese ingredients with one or more of the optional dairy ingredients into a homogeneous plastic mass. One or more of the optional ingredients specified may be used. During its preparation, a pasteurized process cheese food is heated for not less than 30 seconds, at a temperature of not less than 150°F. When tested for phosphatase, the moisture content of a pasteurized process cheese food is not more than 44% and the fat content is not less than 23%.

Pasteurized Process Cheese Spread

Pasteurized process cheese spread is the food prepared by comminuting and mixing, with the aid of heat, one or more of the optional cheese ingredients with or without one or more of the optional dairy ingredients with one or more of the emulsifier into a homogeneous plastic mass, which is spreadable at 70°F.

During its preparation, a pasteurized process cheese spread is heated for not less than 30 seconds at a temperature of not less than 150°F. The moisture content of a pasteurized process cheese spread is more than 44% but not more than 60%, and the milk fat content is not less than 20%.

Provolone Cheese

Provolone, a pasta filata or stretched curd-type cheese which produces a finished cheese having minimum milk fat content, is 45% by weight of the solids and the maximum moisture content is 45% by weight. If the dairy ingredients used are not pasteurized, the cheese is cured at a temperature of not less than 35°F for at least 60 days.

Romano Cheese

Romano cheese is the food prepared from the milk of cow or sheep or goat, or mixtures of two or all of these and other ingredients. It grates readily, and has a granular texture and a hard and brittle rind. It contains not more than 34% of moisture, and its solids contain not less than 38% of milk fat, it is cured for not less than 5 months.

Roquefort Cheese, Sheep's Milk Blue Mold, and Blue-Mold Cheese from Sheep's Milk

Roquefort cheese, sheep's milk blue-mold cheese, blue-mold cheese from sheep's milk, is characterized by the presence of bluish-green mold, *Penicillium*

roquefortii, throughout the cheese. The minimum milk fat content is 50% by weight of the solids and the maximum moisture content is 45% by weight. The dairy ingredients used may be pasteurized. Roquefort cheese is at least 60 days old.

Samsoe Cheese

It has a small amount of eye formation of approximately uniform size of about five-sixteenths inch (8 mm). The minimum milk fat content is 45% by weight of the solids and the maximum moisture content is 41% by weight. The dairy ingredients used may be pasteurized. Samsoe cheese is cured at not less than 35°F for at least 60 days.

Sapsago Cheese

The cheese is pale green in color and has the shape of a truncated cone. The maximum moisture content is 38% by weight. Sapsago cheese is not less than 5 months old.

Swiss or Ementaler Cheese

It has holes or eyes developed throughout the cheese. The minimum milk fat content is 43% by weight of the solids and the maximum moisture content is 41% by weight. The dairy ingredients used may be pasteurized. Swiss cheese is at least 60 days old.

ICE CREAM AND FROZEN DESSERTS

According to the FDA, ice cream is a food produced by freezing, while stirring, a pasteurized mix consisting of one or more of the dairy ingredients and other safe and suitable ingredients added to achieve their intended effect. Ice cream contains not less than 1.6 lb of food solids to the gallon and weighs not less than 4.5 lb to the gallon. Ice cream contains not less than 10% milk fat, nor less than 20% total milk solids.

In the United States, a product can be called ice cream only when it contains milk fat. In other parts of the world, such a stringent requirement for milk fat does not exist and vegetable fats are often used. Additionally, in other parts of the world, the minimum amount of fat required for a product to be called ice cream can be below the 10% minimum required in the United States. The NLEA fully implemented in 1995 allowed for some modifying terminology. According to this act, ice cream can be modified by the removal of fat to be called reduced fat ice cream, light

ice cream, low-fat ice cream, and no-fat ice cream. In order to understand these modified terms, it is necessary to realize that NLEA was promulgated to aid food processors to make foods that would result in lowering the fat intake of the U.S. population without having to come up with names for foods that consumers did not like. Reduced fat ice cream should achieve a 25% reduction in fat over the full-fat counterpart. This means ice cream with 7.5% fat can be labeled as reduced fat. Light ice cream should result in a 50% reduction in total fat and a 33% reduction in calories. According to this definition a 5% fat ice cream with a 33% reduction in calories can be labeled as light ice cream. Low-fat ice cream should provide less than 3 g of fat per serving of ice cream. One serving of ice cream is considered to be one half cup (a volumetric measure). Finally, no fat ice cream is an ice cream that contains less than half a gram of fat per serving. These modified ice creams can also weigh not less than 4.0 lb per gallon rather than the 4.5 lb per gallon stipulated for regular ice cream.

Frozen custard or French style ice cream meets all standards for ice cream and must contain a minimum of 1.5% egg-yolk solids. Pasteurized frozen sugared egg yolks or dried egg yolks are used as ingredients. Fresh pasteurized egg yolks may also be used in frozen custard manufacture.

There are other frozen desserts that also have a standard of identity. These are sherbet, mellorine, and water ice. Sherbet should contain not less than 1% milk fat and not more than 2% milk fat; MSNF content should be not less than 2% and not more than 5%. The minimum weight requirement is 6 lb per gallon. A fruit flavored sherbet should have a minimum acidity of 0.35%. Mellorine is a product made with fats other than milk fat. These fats can be animal- or vegetable-derived. Mellorine should contain a minimum of 1.6 lb of food solids per gallon, a fat content of not less than 6%, a minimum protein content of 2.7% (the protein has to be of equal nutritional value of milk protein), and a gallon of mellorine should weigh not less than 4.5 lb per gallon. Water ices have the same standards as sherbet except no milk or egg ingredient is allowed (except egg white). The terms sorbet and frozen yogurt are also used and these have no federal standards of identity but some states have established standards for frozen yogurt.

Optional Ingredients

Permitted optional ingredients in ice cream and frozen desserts are cream; dried cream; plastic cream (sometimes known as concentrated milk fat); butter;

butter oil; milk; concentrated milk; evaporated milk; sweetened condensed milk; superheated condensed milk; dried milk; skim milk; concentrated skim milk; evaporated skim milk; condensed skim milk; superheated condensed skim milk; sweetened condensed skim milk; sweetened condensed part-skim milk; nonfat dry milk; sweet cream buttermilk; condensed sweet cream buttermilk; dried sweet cream buttermilk; skim milk, that may be concentrated, and from which part or all the lactose has been removed by a safe and suitable procedure; skim milk in concentrated or dried form that has been modified by treating the concentrated skim milk with calcium hydroxide and disodium phosphate; and whey and those modified whey products (e.g., reduced lactose whey, reduced minerals whey, and whey protein concentrate) that have been determined by FDA to be generally recognized as safe (GRAS) for use in this type of food. Water may be added, or water may be evaporated from the mix. The sweet cream buttermilk and the concentrated sweet cream buttermilk or dried sweet cream buttermilk, when adjusted with water to a total solids content of 8.5%, has a titratable acidity of not more than 0.17%, calculated as lactic acid. The term "milk" as used in this section means cow's milk. Any whey and modified whey products used contribute, singly or in combination, not more than 25% by weight of the total nonfat milk solids content of the finished food. The modified skim milk, when adjusted with water to a total solids content of 9%, is substantially free of lactic acid as determined by titration with 0.1N NaOH, and it has a pH value in the range of 8.0–8.3. The caseinates that may be added to ice cream mix containing not less than 20% total milk solids are casein prepared by precipitation with gums, ammonium caseinate, calcium caseinate, potassium caseinate, and sodium caseinate. Caseinate may be added in liquid or dry form, but must be free of excess alkali. Optional hydrolyzed milk proteins may be added as stabilizers at a level not to exceed 3% by weight of ice cream mix containing not less than 20% total milk solids, provided that any whey and modified whey products used contribute, singly or in combination, not more than 25% by weight of the total nonfat milk solids content of the finished food.

Processing

Products frozen without agitation are called quiescently frozen. Therefore, ice cream mix frozen without agitation is labeled as quiescently frozen dairy

confection. Water ice frozen without agitation is labeled as quiescently frozen water ice or iced confection.

All types of mix (excluding water ice mix) have to be pasteurized. Because of the higher solids levels in these mixes, when compared to milk, the proscribed time and temperature of pasteurization are 155°F for 30 minutes or 175°F for 25 seconds. Pasteurization has to be accomplished by using properly designed and operated and certified equipment.

FOOD ADDITIVES AND PACKAGING

Ingredients and food compounds that are added to milk and fermented milk products must be safe and suitable for their intended function. The FDA reviews the safety of food and color additives before manufacturers and distributors can market them. To initiate this review, food additive firms are required to submit a petition or notification that includes appropriate test data to demonstrate the safety of the intended use of the substance. The agency also has a notification program for substances that are "GRAS". Ingredient manufacturers can self-affirm their product to be GRAS as long as they have scientific proof (published studies) to support such affirmation. These data have to be submitted to FDA to substantiate the self-affirmed GRAS status of the ingredient.

Food packaging is regulated as a food-contact surface. The FDA defines a food-contact substance as "any substance intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use is not intended to have a technical effect in such food." Safety evaluations of food-contact surfaces are done by an FDA notification process to authorize new uses of food additives that are food-contact substances based on a detailed analysis of the compounds' chemistry, toxicology, and environmental impact. An inventory of effective notification for food-contact substances and additional information regarding the notification program are listed on FDA's web page <http://www.cfsan.fda.gov/~dms/opa-fcn.html>.

An informational database on approved food additives is maintained by the FDA. It contains administrative, chemical, and toxicological information on thousands of substances directly added to food, including substances regulated by the FDA as direct, "secondary" direct, color additives, GRAS, and prior-sanctioned substances. More than 3,000 total substances together comprise an inventory often referred to as "*Everything Added to Food in the United*

States (EAFUS). This information can be found at <http://www.cfsan.fda.gov/~dms/eafus.html>.

LABELING

FDA sets forth general requirements for food labeling in the FDA Federal Food Drug and Cosmetic Act (FFD&C Act) and more detailed regulations in the CFR Title 21 Parts 100. The basic premise is that food labels must be truthful and not misleading to consumers. The NLEA of 1990 established most federal food labeling requirements as nationally uniform standards through federal preemption of state requirements. Under the preemptive authority of the NLEA, no state can directly or indirectly establish or continue to enforce any requirement that is not identical to a federal requirement issued under the following provisions of the FFD&C Act.

Any product introduced into interstate commerce is subject to FDA regulations. The U.S. Court has interpreted the scope of interstate commerce expansively for this purpose so as to apply federal regulation to virtually all products except those for which the product, including its ingredients and packaging materials, are produced, packaged, and sold within the given state. As a result, there are few “intrastate” products, but those that qualify are not subject to FDA regulation.

There are several types of state food labeling requirements that are not expressly preempted, and thus, may be enforced against food products in interstate commerce. These include warning labels, open date coding, unit price labeling, food grading, recyclable container deposit labeling, religious dietary labeling, and item price labeling.

While federal standards of identity preempt state standards, states may continue to establish and enforce standards of identity for products for which no federal standards of identity exist, for example, frozen yogurt, milk beverages, yogurt smoothies, or feta cheese.

General Requirements

The food labeling regulations specify what information must appear on the package label, where information must be placed, the label format, and the size for mandatory labeling material such as nutrition information. The part of the package that the consumer is most likely to see during normal retail display is called the “principal display panel.” Information related to the product name including flavoring and the

net quantity expressed by weight or volume must appear in the food product’s principal display panel. The “information panel” is typically located to the right of the principal display panel and must contain the full ingredient listing, name, and place of business of manufacturer, packer, or distributor, nutrition labeling of food, and, if applicable, specific requirements related to the use of nutrient content claims, food warnings, and statements of special dietary use.

In addition to the general labeling requirements of the federal regulations, the vast majority of states will mandate the inclusion of additional labeling as required by the Grade “A” PMO discussed in Chapter 3. The Grade “A” PMO’s labeling requirements call for all bottles, containers, and packages containing milk and milk products to be conspicuously marked with the term “Grade A”, identity of the plant where pasteurized, identification of processing if “ultra-pasteurized” or “aseptic,” “reconstituted,” or “recombined” if the product is made by reconstitution or recombination, and the terms “keep refrigerated after opening” in the case of aseptically processed milk and milk products.

Product—Nomenclature

The name of the food or “statement of identity” may be established by regulation, or it may be dictated in the nomenclature section of the product’s standard of identity. If the product does not fall under a federal standard or, as applicable, state standard of identity or common or usual name, then an appropriate descriptive name must be used that will easily be understood by consumers. The standards of identity for many milk products, cheeses, and frozen desserts designate the name of the product. Descriptive names may only be used on a product which does not have a standard of identity, or common or usual name. A descriptive name must be suggestive enough to reveal the basic composition of the product and alleviate any question regarding the product’s identity. For example, a beverage product made of a blend of yogurt and juice should not solely use the name “smoothie” but included that it is “a blend of yogurt and juice.” In addition, the form of the food such as slices, cubes, or liquids must be stated if it is not visible through the packaging. For example, drinkable yogurt would not require a disclosure that the product is a liquid rather than a semisolid if it is packaged in a transparent container where the consumer can clearly see the viscosity or form of the food.

Flavor Labeling

Milk, milk products, yogurt, and frozen desserts are labeled with the name of the food and the flavoring, if added. Flavorings are defined by FDA as either natural or artificial. Artificial flavors are compounds that impart flavor which is *not* derived from a spice, fruit or fruit juice, vegetable or vegetable juice, yeast, herb, bark, bud, root, leaf, or plant material, meat, seafood, poultry, eggs, dairy products, and fermented products. Natural flavor or natural flavoring is derived from the compounds listed above in the form of an essential oil, oleoresin or extract, protein, hydrolysate, distillate that is used to impart flavor.

Flavor labeling for all foods and dairy products except ice cream is dictated by FDA food labeling regulation according to the general “6-Category” flavor labeling system. The 6-Category flavor labeling categories will be referred to as Category A through Category F (IDFA, 2004).

The first three categories (A, B, and C) apply when a flavor, including artificial flavor, is added to a food product in fluid form or “from the bottle” (e.g., vanilla extract, vanillin, coffee extract).

Category A. When the primary characterizing flavor ingredient is solely natural, not artificial and is derived from the product whose flavor it simulates, resembles, or reinforces, the name of the food is accompanied by the common or unusual name of the characterizing flavor (e.g., “Vanilla yogurt”).

Category B. When the food contains both natural flavor derived from the characterizing flavor source and other natural flavoring from a source that simulates, resembles, or reinforces the characterizing flavor, the name of the food is followed by the words “with other natural flavor” (e.g., “Coffee yogurt with other natural flavor”).

Category C. When natural flavor(s) used in the food is not derived from the ingredient whose flavor has been determined to be the characterizing flavor or if the food contains an artificial flavor which simulates, resembles, or reinforces the declared characterizing flavor, the name of the food must be accompanied by the words “artificial or artificially flavored” (e.g., “Artificially flavored vanilla yogurt”).

The next two categories (D and E) apply to those products that consumers would commonly expect to contain the characterizing food ingredient(s) (e.g.,

strawberries, blueberries). In both of these categories, the characterizing food ingredient(s) is added to flavor the finished product at a level *not* sufficient to independently characterize the finished product.

Category D. When the food contains an *insufficient* amount of the food ingredient to independently characterize the product, and it contains added natural flavor which is derived from the characterizing food ingredient, the food is labeled as a naturally flavored food. The flavor *may* be immediately preceded by the word “natural” and must be immediately followed by the word “flavored” (e.g., “Peach flavored yogurt” or “Natural peach flavored yogurt”).

Category E. When the food contains an *insufficient* amount of the food ingredient to independently characterize the food and it contains other added natural flavor(s) which are not derived from the characterizing flavor declared on the label, but which simulate, resemble, or reinforce the characterizing flavor, the flavor *may* be immediately preceded by the word “natural” and must be immediately followed by the words “with other natural flavors” (e.g., “Peach yogurt with other natural flavors” or “Natural peach yogurt with other natural flavors”).

Category F applies to products that contain sufficient amounts of characterizing food ingredients to flavor the finished product (e.g., peaches, cherries).

Category F. If the food contains *sufficient* levels of the food ingredient to independently characterize the food and contains no added artificial flavors or natural flavors (“from the bottle”) which simulate, resemble, or reinforce the characterizing flavor, then the characterizing ingredient is the flavor of the food (e.g., “Strawberry yogurt”).

The name of the flavoring as described above must accompany the name of the food on the principal display panel of the package or any panels where the product name occurs. A blend of three or more distinctive artificial flavors can be described as a collective name, that is, “Artificially Flavored Tutti Fruity.” The name of the flavoring must be in a type size not less than 1/2 the height of the letter used in the name of the food and the flavor modifying terms must be not less than 1/2 the height of the name of the characterizing flavor. Exemptions for category name declaration are made if the flavor name is part of a trademark such as “Lemon DropTM.”

Table 6.1. Common or Usual Names for Typical Ingredients Used in Dairy Products

Ingredient	Common or Usual Name
Skim milk, concentrated skim milk, reconstituted skim milk, and nonfat dry milk (21 CFR §101.4 <i>Food; designation of ingredients</i>)	“Skim milk” or “nonfat milk”
Milk, concentrated milk, reconstituted milk, and dry whole milk (21 CFR §133.129 <i>Dry curd cottage cheese</i> , 21 CFR §101.4 <i>Food; designation of ingredients</i>)	“Milk”
Bacteria cultures (21 CFR §131.160 <i>Sour cream</i> , 21 CFR §131.162 <i>Acidified sour cream</i> , 21 CFR §101.4 <i>Food; designation of ingredients</i>)	“Cultured —” (the blank is filled in with the name of the substrate)
Sweet cream buttermilk, concentrated sweet cream buttermilk, reconstituted sweet cream buttermilk and dried sweet cream buttermilk (21 CFR §101.4 <i>Food; designation of ingredients</i>)	“Buttermilk”
Whey, concentrated whey, reconstituted whey, and dried whey (21 CFR §101.4 <i>Food; designation of ingredients</i>)	“Whey”
Cream, reconstituted cream, dried cream, and plastic cream (sometimes known as concentrated milk fat) (21 CFR §101.4 <i>Food; designation of ingredients</i>)	“Cream”
Butter oil and anhydrous butterfat (21 CFR §101.4 <i>Food; designation of ingredients</i>)	“Butterfat”
Milk-clotting enzymes (21 CFR §133.128 <i>Cottage cheese</i> , 21 CFR §133.129 <i>Dry curd cottage cheese</i>)	“Enzymes”

Source: IDFA, 2004.

For ice cream, a three-category flavor labeling system is used. Category I ice cream contains all natural flavors and is labeled as Vanilla ice cream if vanilla is the characterizing flavor. If a mixture of natural and artificial flavors in which the natural flavor predominated the product will be labeled as vanilla-flavored ice cream (if vanilla is the characterizing flavor). Such products are called Category II while all other ice cream products that do not conform to flavor requirements of Categories I and II are labeled as Category III and the product must clearly state Artificially flavored vanilla ice cream if vanilla is the characterizing flavor.

Ingredient Declaration

An ingredient statement is required on all food packages intended for retail sale that contain more than one ingredient. Except where exemptions are applicable, an individual ingredient must be declared in the ingredient statement by its common or usual name. In addition, specific regulations exist for colors, sweeteners, incidental additives, processing aids, fat and/or

oil, and allergenic ingredients. Special ingredient labeling situations include the following:

All certified colors must be included by name in the ingredient statement.

Any beverage product purporting to contain fruit or vegetable juice must declare the percent of juice present in the finished product.

Many standards of identity address ingredient labeling, in that they allow for ingredient groupings or provide a common or usual name for a particular ingredient. Some examples are listed in Table 6.1.

The ingredient listing must appear prominently and conspicuously on either the principal display panel or the information panel. The entire list of ingredients must appear in one place without other “intervening material” and, in general, must appear in letters not less than 1/16 of an inch in height.

Ingredients in multicomponent foods may be listed by either of the following two alternatives: grouping or dispersion. Although either method may be used, the grouping alternative may be more helpful to consumers in identifying the ingredients used in each component of the food.

The grouping alternative for ingredient declarations of multicomponent ingredients may be used by declaring the common or usual name of the ingredient followed by a parenthetical listing of all ingredients contained in each of the components in descending order of predominance by weight. For example, an ingredient statement for raspberry yogurt with granola may state:

Ingredients: yogurt (cultured milk, raspberries, sugar, gelatin, pectin and natural flavors) and granola topping (rolled oats, puffed rice, corn syrup, brown sugar, raisins, and almonds).

The dispersion alternative for ingredient declarations of multicomponent ingredients may be used by incorporating into the ingredient statement (in descending order of predominance in the finished food), the common or usual name of every component of the multicomponent ingredient without listing the multicomponent ingredient itself. For example, an ingredient statement for raspberry yogurt with granola may state:

Ingredients: cultured milk, sugar, rolled oats, corn syrup, raspberries, puffed rice, brown sugar, raisins, almonds, gelatin, pectin and natural flavors.

Nutrition Facts Panel

All food packages intended for retail sale must declare quantitative nutritional information expressed in terms of a “serving” of an individual food. A “serving,” or as it appears on the label, “Serving Size,” is based on the reference amount of food customarily consumed per eating occasion by persons 4 years of age or older as expressed by a common household measure appropriate for the food.

FDA has established reference amounts for over 100 food product categories. The established reference amount is the benchmark for determining the serving size declared on the label and expressed as a common household measure (e.g., cups, tablespoons, teaspoons). The serving size is required to be expressed on the nutrition label in common household measure followed in parentheses by an equivalent metric quantity (fluid products in milliliters and all other foods in grams). For acidophilus milk, as an example, “Serving Size 1 cup (240 mL).” For the most part, the common household unit for similar products will be the same, but because of the varying densities among products, the metric equivalent may not be identical.

Table 6.2. Reference Amount Categories for Milk and Milk Products

Product Category	Reference Amount
Cheese used as an ingredient (e.g., dry cottage cheese)	55 g
Sour cream	30 g
Milk and cultured or acidified milk	240 mL
Yogurt	225 g
Dairy-based dips	2 tbsp
Dairy and nondairy whipped topping	2 tbsp
Juices, juice drinks, and juice milk blend drinks	240 mL
Shakes or shake substitutes (e.g., dairy shake mixes)	240 mL

Unless otherwise exempted, all nutrients and food component quantities must be declared on the basis of the serving size derived from the reference amount. FDA has established methods for converting the reference amount to the “serving size” for labeling purposes. The method employed is based on the type of container in use (i.e., multiserving vs. single-serving container) and the physical characteristics of the product (discrete unit vs. nondiscrete fluid or bulk-type product).

For example, manufacturers producing frozen yogurt mix for retail sale must determine the amount of mix that will make (under normal conditions of preparation) 1/2 cup of product. Since air (i.e., overrun) is incorporated into the product, less than 1/2 cup of mix will be required to produce 1/2 cup of finished product. Table 6.2 shows reference amount categories that have been established for milk and milk products. Nutrition information is presented to consumers “in the context of a total daily diet,” which is mandated by regulations as a diet of 2,000 calories per day. From this theoretical 2,000 calorie per day diet, recommended intake levels or “Daily Values” (DV) of individual nutrients have been developed on the basis of current dietary guidelines. As a result, information on individual nutrients is required to be expressed in most cases by a quantitative declaration (grams, milligrams, etc.) and a percentage of a DV for the nutrient.

Nutrient labeling information is referred to as the Nutrition Facts box. The explicit amount (quantitative declaration) and, as applicable, the percentage of

Table 6.3. Daily Reference Values for Key Nutrients Based on a 2,000-Calorie Diet.

Food Component	Daily Reference Values
Total fat	65 g
Saturated fat	20 g
Cholesterol	300 mg
Sodium	2,400 mg
Potassium	3,500 mg
Total carbohydrate	300 g
Dietary fiber	25 g
Protein	50 g

Table 6.4. Recommended Daily Intake Values for Some Micronutrients.

Nutrient	RDI Value
Vitamin A	5,000 IU
Vitamin C (ascorbic acid)	60 mg
Calcium	1,000 mg
Iron	18 mg
Vitamin D	400 IU

the DV must be included in the Nutrition Facts box for each of the following nutrients and food components:

Total calories	Dietary fiber
Calories from fat	Sugars
Total fat	Protein
Saturated fat	Vitamin A
Trans fat	Vitamin C
Cholesterol	Calcium
Sodium	Iron
Total carbohydrate	

Table 6.3 shows a list of the daily reference values (DRV) based on a 2,000-calorie diet.

The percent DV is calculated by dividing the unrounded (actual amount) or rounded amount of the nutrient present in the food per serving by the established DRV and multiplying by 100, except that the DRV for protein must be calculated from the unrounded amount. The DV is expressed to the nearest whole percentage. The percentage of DV is mandated for the following nutrients: total fat, saturated fat, cholesterol, sodium, total carbohydrates, and dietary fiber and is voluntary for potassium and protein. There has been no DV set for trans-fat so a percentage of DV declaration should not be made (see Table 6.4).

Nutrition Facts			
Serving size 1 cup (228 g)			
Servings per container 2			
Amount per serving			
Calories 260		Calories from fat 120	
		% Daily value*	
Total fat	13 g	20%	
Saturated fat	5 g	25%	
Trans fat	2 g		
Cholesterol	30 mg	10%	
Sodium	660 mg	28%	
Total carbohydrate	31 g	10%	
Dietary fiber	0 g	0%	
Sugars	5 g		
Protein	5 g		
Vitamin A 4% • Vitamin C 2%			
Calcium 15%		Iron 4%	
*Percent Daily Values are based on a 2,000 calorie diet, Your Daily Values may be higher or lower depending on your calorie needs:			
	Calories:	2,000	2,500
Total fat	Less than	65 g	80 g
Sat. fat	Less than	20 g	25 g
Cholesterol	Less than	300 mg	300 mg
Sodium	Less than	2,400 mg	2,400 mg
Total carbohydrate		300 g	375 g
Dietary fiber		25 g	30 g
Calories per gram:			
Fat 9	*	Carbohydrate 4	* Protein 4

Figure 6.1. Full vertical format of a nutrient label.

Depending on the size of the package, FDA allows different graphic formats for nutritional information. The most common is the full vertical format used on all packages with greater than 40 in.² of available labeling space (see Fig. 6.1).

Packages with less than 40 in.² of labeling space can use a smaller tabular format (see Fig. 6.2).

Special Labeling Requirements. Food labeling often has additional nonmandatory information used for marketing purposes these are listed below.

Real seal. Dairy Management, Inc., has established a voluntary program to promote dairy products and to distinguish between authentic and simulated dairy products. They have chosen the “REAL®” seal to

Figure 6.2. Alternate tabular format for nutritional label.

indicate this distinction. Use of the “REAL®” seal must be in conjunction with the “REAL®” Seal Certificate User Agreement. Information about the seal may be obtained at <http://www.dairyinfo.com>.

Live and active cultures seal. To help identify yogurt products that contain live and active cultures, the NYA has established a special *Live & Active Cultures* seal. The NYA is a national nonprofit trade organization whose purpose is to sponsor health and medical research for yogurt with live and active cultures and serve as an information source to the trade and the general public. The *Live & Active Cultures* seal, which appears on refrigerated and frozen yogurt containers, helps identify those products containing significant amounts of live and active cultures. The seal is a voluntary identification available to all manufacturers whose refrigerated yogurt contain at least 100 million cultures per gram at the time of manufacture, and whose frozen yogurt contains at least 10 million cultures per gram at the time of manufacture. Since the seal program is voluntary, some yogurt products may have some live cultures but not carry the seal. More information can be found at <http://www.aboutyogurt.com>.

Kosher symbols. Observance of the biblical kosher laws can be facilitated by kosher foods being certified by a rabbinical organization and labeled with an identifying symbol. The bible lists certain basic categories of food items which are not kosher. These include certain animals, fowl, and fish (such as pork and rabbit, eagle and owl, catfish and sturgeon) and any shellfish, insect, or reptile. In addition, kosher species of meat and fowl must be slaughtered in a prescribed manner and meat and dairy products may not be manufactured or consumed together. Kosher

food labeling regulations are not preempted by the implementation of the NLEA and, therefore, state regulatory officials can enforce their own state regulations. Although FDA does not discuss the criteria by which these terms, “kosher” and “kosher style,” may be used, they do indicate that these terms should only be used on products that meet the religious dietary requirements.

More information on kosher certification can be obtained by contacting the following organizations: the Union of Orthodox Jewish Congregations, in New York, NY, at <http://www.oukasher.org> or the OK Kosher Certification at <http://www.okkasher.com>.

Universal product bar codes. The Uniform Code Council was originally created by the food industry in an effort to place a code and scanner-readable symbol on the package of containers sold through retail outlets using automatic checkout equipment. The primary purpose of the Universal Product Code (UPC) bar code system is to reduce retail store costs by providing an automatic computerized checkout system, to establish better inventory control and ordering systems, and to provide more valuable marketing information about products. A UPC manufacturer identification number for use in the bar code may be obtained by contacting the Uniform Code Council, Inc., in Dayton, OH, or web site <http://www.uc-council.org>.

Code dating. Code dating, such as “sell by” or “best if used by” dating, is a requirement promulgated under the state regulations and laws and enforced by state regulatory officials. There are no federal regulations addressing code dating or “sell by” dating. Often a code date printed on the food label is used for tracking and identifying the food by the date of production, plant location, fill line, or

Table 6.5. Culture Characterization for Codex Standard for Fermented Milk

Yogurt	Symbiotic cultures of <i>Streptococcus thermophilus</i> and <i>Lactobacillus delbrueckii</i> subsp. <i>Bulgaricus</i>
Alternate culture yogurt	Cultures of <i>Streptococcus thermophilus</i> and any <i>Lactobacillus</i> species
Acidophilus milk	<i>Lactobacillus acidophilus</i>
Kefi	Starter culture prepared from kefi grains, <i>Lactobacillus kefir</i> , species of the genera <i>Leuconostoc</i> , <i>Lactococcus</i> , and <i>Acetobacter</i> growing in a strong specific relationship. Kefi grains constitute both lactose fermenting yeasts (<i>Kluyveromyces marxianus</i>) and non-lactose-fermenting yeasts (<i>Saccharomyces unisporus</i> , <i>Saccharomyces cerevisiae</i> , and <i>Saccharomyces exiguus</i>)
Kumys	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> and <i>Kluyveromyces marxianus</i>

Note: Microorganisms other than those constituting the specific starter culture(s) specified above may be added.

Source: Codex Standard for Fermented Milk (243-2003).

production vat. This information may be used for inventory purposes, product rotation in storage, display and, if necessary, retrieval from the market. Therefore, it is important that the code date or identifying information be legible printed on each container and shipper.

Food warning statements. FDA regulations require food-warning statements to appear in the labeling of certain food products. For example, the regulations pertaining to the use of aspartame in a food product require that the label state on either the principal display panel or the informational panel the following: “Phenylketonurics: Contains Phenylalanine.”

Codex Standards and Definition for Fermented Milk Products

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) developed the Codex Alimentarius Commission—the body charged with developing a worldwide food code. All important aspects of food pertaining to the protection of consumer health and fair practices in the food trade have come under the Commission’s scrutiny, including international food standards, also known as Codex Standards. The Codex web site lists more information on the Codex Alimentarius and official Codex Standards.

The *Codex Standard For Fermented Milk* (243-2003), recently updated in 2003, applies to all fermented milk including heat-treated fermented milks, concentrated fermented milks, and composite fermented milks (fermented milks with flavoring or

other added nondairy ingredients) that are both directly consumed or used for further processing. The Codex fermented milk standard also provides that certain fermented milk must be characterized by specific starter cultures (see Table 6.5; Codex, 2004).

Concentrated fermented milk such as strained yogurt, Labneh, Ymer, and Ylette require that the protein be increased to 5.6%. Flavored fermented milk must contain a maximum of 50% (mass/mass) of nondairy ingredients, such as sweeteners, fruits, vegetables, juices, purees, cereals, nuts, spices, and other natural flavorings.

Raw materials allowed in the Codex Standard for Fermented Milks are limited to milk and/or milk products obtained from milk and potable water used for reconstitution. Additional permitted ingredients include starter cultures and sodium chloride. Gelatin and starch are allowed only in heat-treated fermented milks, flavored fermented milks, and plain fermented milk if permitted by the regulations in the country of sale to the final consumer.

Composition requirements for various Codex Fermented Milks are listed in Table 6.6.

The microbial criteria apply to the fermented milk portion only for flavored fermented milks. Compliance to the microbial criteria is verified through analytical testing of the product through the end of the shelf life on products that have been stored under normal conditions and temperatures.

Allowable food additives are specified in Table 6.7.

Labeling of the product is also specified by the Codex Standard for Fermented Milks. It allows for names to be replaced by designations such as

Table 6.6. Composition Requirements for Codex Standard for Fermented Milk

	Fermented Milk	Yogurt, Alternate Culture Yogurt and Acidophilus Milk	Kefi	Kumys
Milk protein ^a (% m/m)	Min. 2.7%	Min. 2.7%	Min. 2.7%	
Milk fat (% m/m)	Less than 10%	Less than 15%	Less than 10%	Less than 10%
Titrateable 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 the starter culture define in section 2.1 (CFU/g, in total)	Min. 10 ⁷	Min. 10 ⁷	Min. 10 ⁷	Min. 10 ⁷
Labeled micororganisms ^b (CFU/g, total)	Min. 10 ⁶	Min. 10 ⁶		
Yeast (CFU/g)			Min. 10 ⁴	Min. 10 ⁴

^aProtein content 6.38 multiplied by the total Kjeldahl nitrogen determined.
^bApplies where a content claim is made in the labeling that refers to the presence of a specifi microorganism (other than those specific in Table 6.5 for the product concerned) that has been added as a supplement to the specifi starter culture.
Source: Codex Standard for Fermented Milk (243-2003).

Yogurt, Kefi , and Kuyums and provide for alternative spelling of the name to be appropriate in the country of retail sale. Additionally, the qualifying labeling terms “milk” or “tangy” can be used. If the fermented milk product is subject to heat-treatment after cultur-

ing, it must be labeled as “Heat-Treated Fermented Milk”; unless the consumer would be misled by this name, the product shall be named as permitted by the regulations in the country of retail sale. Flavor designations and the term “sweetened,” if appropriate,

Table 6.7. Allowable Food Additives for Codex Standard for Fermented Milk

Additive class	Fermented Milks		Fermented Milks Heat-Treated After Fermentation	
	Plain	Flavored	Plain	Flavored
Colors	—	X	—	X
Sweeteners	—	X	—	X
Emulsifier	—	X	—	X
Flavor enhancers	—	X	—	X
Acids	—	X	X	X
Acidity regulators	—	X	X	X
Stabilizers	X ¹	X	X	X
Thickeners	X ¹	X	X	X
Preservatives	—	—	—	X
Packaging gases	—	X	X	X

X, The use of additive belonging to the class is technologically justified In the case of fl vored products, the additive is technologically justifie in the dairy portion; —, the use of additives belonging to the class is not technologically justified ¹, use is restricted to reconstitution and recombination and if permitted by national legislation in the country of sale to the fina consumer.
Source: Codex Standard for Fermented Milk (243-2003).

shall also be included on the label. A declaration on milk fat content either in percentage or grams per serving should be provided if the consumer would be misled by its omission.

GLOSSARY

ASEPTICALLY PROCESSED—When used to describe a milk product, the product has been subjected to sufficient heat processing and packaged in a hermetically sealed container, to conform to the applicable requirements of the Code of Federal Regulations and the Grade “A” PMO, and to maintain the commercial sterility of the product under normal nonrefrigerated conditions.

CERTIFIED COLORS—Color additives manufactured from petroleum and coal sources listed in the Code of Federal Regulations for use in foods, drugs, cosmetics, and medical devices.

COMMON OR USUAL NAME—The name of a food that is not set by law or regulation, but either through common usage or through expert opinion (such as that of the FDA).

CODEX ALIMENTARIUS COMMISSION—An international body, created by FAO and WHO, to develop food standards, guidelines, and related texts such as codes of practice. The main purposes are protecting health of consumers and insuring fair-trade practices in the food industry, and promoting coordination of all food standards work undertaken by international governmental and non-governmental organizations.

DAILY REFERENCE VALUES (DRV)—An amount set by the CFR as the recommended level of intake for certain nutrients (fat, saturated fat, cholesterol, total carbohydrate, fiber, sodium, potassium, and protein) based on a 2,000-calorie diet.

RDI (REFERENCE DAILY INTAKE)—A value set by the CFR as the recommended level of intake for vitamins and minerals essential for human nutrition for adults and children of 4 or more years of age.

CFU/g (COLONY FORMING UNITS PER GRAM)—An expression of measurement for determining the number of live microorganisms on a volume basis.

FDA—U.S. Food and Drug Administration.

FFD&CA (FEDERAL FOOD DRUG AND COSMETIC ACT)—An act of the U.S. Congress that specifies the basis for food safety standards.

GRADE “A” PMO (PASTEURIZED MILK ORDINANCE)—Model milk regulations used for the inspection of milk production and processing facilities.

HOMOGENIZATION—Mechanical process of shearing milk fat globules via pressure to reduce the size of the fat globules and reduce the separation of the cream portion of the product.

IU (INTERNATIONAL UNITS)—A unit of measurement for certain vitamins (vitamins A, D, and K) for labeling purposes.

IDFA (INTERNATIONAL DAIRY FOOD ASSOCIATION)—A trade association representing dairy processors that provides information and publications on dairy product regulations and standards.

KJELDAHL—Chemical analysis method used to determine the protein content of milk by nitrogen determination.

MSNF—Milk solids-not-fat portion of milk or milk products.

PASTEURIZATION—A process of heating fluid milk products to render them safe for human consumption by destroying the disease-producing organisms (pathogens). The process inactivates approximately 95% of all microorganisms in milk.

REFERENCE AMOUNT (REFERENCE AMOUNT CUSTOMARILY CONSUMED)—Values set by the CFR to reflect the amount of a particular food usually consumed per eating occasion by people of 4 years of age or older, based on the major intended use of that food.

TITRATABLE ACIDITY—The measurement of the extent of growth of acid-producing bacteria by determining the lactic acid present in a food through reacting the lactic acid with a standard solution of alkali.

UHT (ULTRAHIGH TEMPERATURE)—Heat treatment at a temperature of 135–150°C for a holding time of 4–15 seconds that sterilizes the product for aseptic packaging to permit storage at ambient temperatures.

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7

Milk from Farm to Plant

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INTRODUCTION

Milk is a highly perishable biological fluid. The composition of milk and the factors that contribute to variability in the composition have been discussed in Chapter 4. Milk from multiple farms is collected in tankers typically every other day and delivered to a processing facility. At this facility (also known as a dairy plant or factory), the milk is stored and processed further to make the appropriate products for which the dairy plant is designed for.

The safety of milk products is of major concern in dairy processing. Hence the regulations for the production and storage of milk at the farm and transportation from farm to the factory, holding and processing required on the factory premises have been promulgated and discussed in this chapter. Regulations also apply for standardized food products that have to meet compositional requirements as well as the use of approved ingredients and processes. These aspects have been discussed in Chapters 8–17. In addition, manufacturers of products may have internal

standards for insuring the quality of the products important to the consumer. Such attributes may include taste, texture, odor, flavor, mouthfeel, color, and keeping quality. These aspects are covered in detail in other chapters.

FROM FARM TO FACTORY

Milk production on the farm is done under strict guidelines that determine its grade (see below). In 2005, the total milk production in the United States was 80.45 billion kg (177 billion pounds). Farms with 200–500 milk animals accounted for approximately 17.5% of the total milk produced. Farms with 50–100 cows and with more than 2,000 cows accounted for 17.4 and 15% of the total milk production, respectively. Also in 2005, 9.041 million cows were tended by 78,295 production units, which results in an average of 115 cows per farm. The general trend in this area is toward less number of farms with larger herd sizes.

Farms utilize various barn and milking designs, either a pipeline in a stanchion barn or free-stall housing with a milking parlors. Cows are milked twice a day in 11 and 13 hours time intervals with a small minority of the herds milking three times a day approximately every 8 hours. The milk from each animal is pumped under vacuum through lines, weighed, and then mixed with milk from other animals in the herd of cows being milked. Milk temperature immediately after milking is approximately at the body temperature of the cow 38°C (100.4°F). At this temperature, many mesophilic microorganisms can grow and therefore to minimize microbial growth, the warm milk is cooled rapidly. Cooling is commonly achieved by plate heat exchangers or by

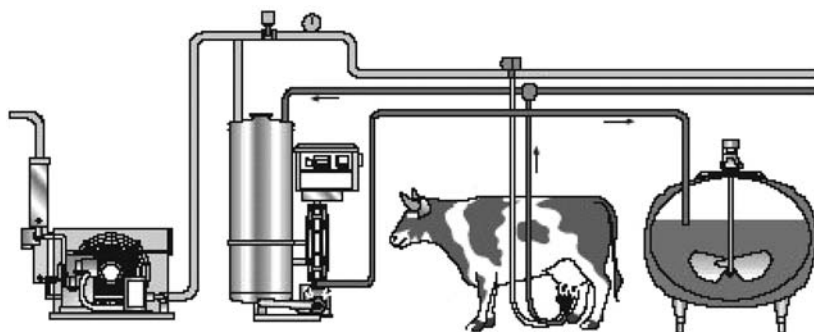


Figure 7.1. Milk from the cow is measured in line and then sent to a bulk-cooling tank. Reproduced with permission from Tetra Pak.

mixing in a refrigerated jacketed bulk storage tank. Milk is collected in insulated tanks called farm bulk milk tanks. Milk from 2 days milking is collected in this tank (Fig. 7.1).

As the number of cows in the herd grows and the number of dairy farms shrinks, milk collection occurs more frequently on the farm. For example, in an Arizona dairy farm, milking 7,000 cows two times a day dispatches a tanker every 45 minutes to their dairy. Smaller farms may use ice bank building tanks. For achieving the best grade of milk (Grade A), milk has to be cooled to below 7°C (45°F) within time limits, for example, 2 hours post milking.

At the time of collecting the milk, the tanker driver obtains a sample for milk from each farm. This sample is the basis for quality determination and for payment based on milk composition.

The tanker itself is made of sanitary stainless steel construction and is fitted with baffle to prevent milk from being vigorously agitated during transportation. Thus churning of milk and the possibility of churning the cream into butter is avoided. At the back end of the tanker is a pump with a volumetric meter and an air-eliminating device. The tanker pulls up to the milk shed at the farm and the driver attaches a sanitary hose to the farm milk storage tank and pumps the milk from the storage bulk tank to the milk transport tanker (Fig. 7.2).

When the farm bulk tank is empty the pump is turned off to prevent air from mixing with milk in the tanker. Presence of air can cause foaming and churning of milk. When the tanker has collected milk from several farms and is full, it is then delivered to a dairy-processing plant of a central milk collection center.

STORAGE OF RAW MILK

Upon arrival of the milk tanker at the dairy, it enters a covered special receiving area. A technician from the quality assurance departments or the plant's milk receiving employee checks the temperature of the milk and draws a representative sample. During this procedure, the sample is checked to determine the odor of the milk and if any off odors are detected that might be unacceptable. The representative sample collected from each tanker is analyzed for sediments, antibiotic residues, somatic cell count, bacteria count, protein and fat content, and freezing point. Some dairies may



Figure 7.2. Collection of milk on the farm. The tanker is pumping milk from the farm bulk milk tank for transport to the dairy factory. Reproduced with permission from Tetra Pak.

also conduct a direct microscopic count of bacteria present in milk. The enumeration methods to determine total bacteria counts and Coliform counts take 24–48 hours. The results of the remaining tests are available within 15–20 minutes. If all tests meet standards set by the dairy, the milk is unloaded from the tanker.

The significance of the receiving dock tests is as follows. Sediment tests point to the quality of milk production at the farm. Antibiotic residue tests indicate if improper withholding time of milk from sick animals treated with antibiotics was commingled with milk from healthy cows. If such commingling occurs, the entire tanker load of milk is rejected. Presence of antibiotics in milk poses a twofold danger. First, antibiotic sensitive individuals could suffer from consuming tainted milk. Second, in the manufacture of cultured milk products, presence of antibiotics may pose a barrier for acidity development by inhibiting the starter culture growth. Somatic cell counts are indicative of general animal health. If they are less than 500,000 per mL of milk, the animal herd health is considered good. If, however, the count exceeds 1,000,000 per mL, it suggests the presence of mastitis or infection in one or more animals in the herd. Mastitic cows are often treated with antibiotics and while receiving the treatment and for a period after the treatment determined by the directions for use, the milk from such animals must be withheld from human consumption, and it is generally discarded on the farm or used for calf feed. Protein and fat contents are used along with the volume of milk received to determine payments and to gain full accounting of raw materials received. This is important for material balance calculations and for determination of losses occurring during processing and packaging. Freezing point of milk is another important test to determine adulteration with water, whether accidental or intentional. Adulteration of milk is a prosecutable offence.

The most common procedure is to record the volume of milk delivered by a tanker. However, in some dairies, the tanker may be weighed prior to emptying and after discharging its load. Volumetric measurements involve a volumetric flow meter fitted with an air eliminator. Presence of air can distort readings of the volume of milk. The milk passes through the air eliminator and a filter into the metering device prior to going to storage silos.

The tanker after discharging its load of milk is cleaned in the receiving bay or in a special cleaning bay. The inside of the tanker is washed by a cleaning in place system which rinses the tanker, cleans it

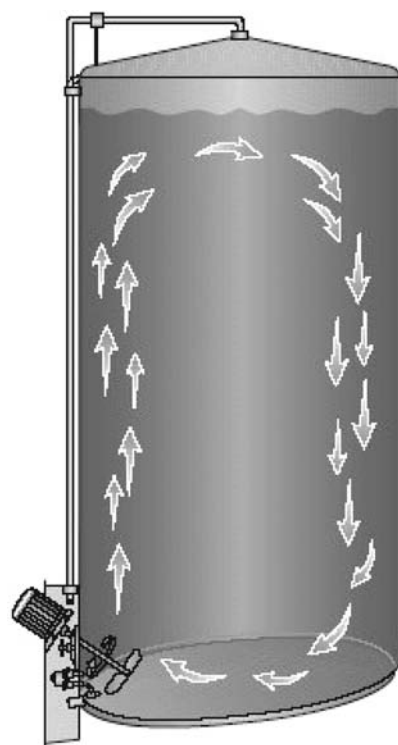


Figure 7.3. Schematic of a milk silo with a propeller agitator. Reproduced with permission from Tetra Pak.

with detergents, and rinses the detergent followed by chemical sanitizing of the tanker. While the inside of the tanker is being cleaned, the exterior is also often washed so that the tankers always look clean on the road. After cleaning and sanitizing the tanker goes to its next round for milk collection.

The raw milk is stored in large vertical tanks known as silos (Fig. 7.3).

These silos can have capacities of 25,000–150,000 liters (6,000–37,000 U.S. gallons). The silos are placed outside the dairy with an inside outlet bay. The silos have double wall construction with an outside welded sheet metal within which a stainless steel tank is contained. The silos have methods of agitating milk so as to prevent gravitational fat separation.

The method of agitation must be gentle to avoid rupture of the milk fat globule membranes which can cause lipolysis of milk fat. Lipolysis generates off flavors and odors. The most common agitation system is to use a mechanical propeller agitator or purged with pressurized filtered air. In the tanks, there are

instruments which include a thermometer, level indicator, low-level protector, overflow protector, and an empty tank indicator. Modern dairies have electronically transmitted data on temperature, levels of milk in the silos, and the protection devices. Redundant visual (nonelectronic) systems may also be employed in some dairies.

Milk storage silos are cleaned in place and visual inspections of the interior surfaces for any problems are also conducted periodically. Since silos are considered confined spaces, entry into a silo has to be strictly according to the standards recommended by Occupational Safety and Health Administration of the U.S. Government.

The temperature of the milk in the silo has to be maintained at 7°C (45°F) or below. Even at these temperatures, psychrotrophic organisms can cause proteolysis and lipolysis if milk is stored for long periods of time. Therefore, it is recommended that the silos be emptied and cleaned and sanitized at regular intervals. The raw milk in the silo is further processed and the main elements in the processing are centrifugal operations, thermal treatment, homogenization, and cooling and packaging.

HISTORY OF MILK SAFETY

Milk is not only rich in nutrients but also has the properties to readily support microbial growth and potentially pathogenic organisms. Milk cows on the farm are exposed to many sources of potential contamination. Some of these may be the water, feed sources, exposure to manure, insects, contact with diseased animals in housing or corral areas, injuries to the udder, and poor milking practices.

Early studies implicate milk as the carrier for many communicable diseases to the consumer. Some of the most notable outbreaks were tuberculosis, brucellosis, salmonellosis, diphtheria, scarlet fever, septic sore throat, and the dysenteries of the food infection type. Recent outbreaks of salmonellosis, listeriosis, yersinia, and campylobacter have been responsible for milk-related human illness. Q fever was also noted as one of the pathogens responsible for milk-borne outbreaks and the imposition of more stringent pasteurization requirements (USDHHS PMO, 2003).

The incidence of milk-borne illness in the United States has been sharply reduced. In 1938, milk-borne outbreaks constituted 25% of all disease outbreaks due to infected foods and contaminated water. Recent information reveals that milk and fluid milk products continue to be associated with less than 1% of such

reported outbreaks. Many groups have contributed to this commendable achievement, including public health and agricultural agencies, dairy and related industries, several interested professional groups, educational institutions, and the consuming public.

The responsibility for insuring the ready availability and safe supply of milk and milk products is the cooperative effort of all engaged, including government regulators and the industry.

United States Public Health Grade “A” Milk Safety Program

The U.S. Public Health Service branch of Food and Drug Administration (FDA) (USPHS) is a division of the Federal Health and Human Services under the FDA, which has a broad authority of overseeing the health and safety of food. The USPHS oversight began at the turn of the century with studies on the role that milk plays in the spread of diseases. The finding indicated that effective public health control of milk-borne disease requires the application of sanitation measures throughout the production, handling, pasteurization, and distribution of milk and milk products. These studies were followed by research to identify and evaluate sanitary measures that might be used to control disease, including studies that led to the improvement of the pasteurization process.

To assist various states and municipalities in initiating and maintaining effective programs for the prevention of milk-borne disease, the USPHS developed a model regulation known as the *Standard Milk Ordinance* for voluntary adoption by State and Local Milk Control Agencies in 1924. An accompanying *Code* was published in 1927 to provide a uniform interpretation of this *Ordinance* and to establish administrative and technical details as to satisfactory compliance. This model milk regulation is still used today though now titled the *Grade “A” Pasteurized Milk Ordinance* (Grade “A” PMO), 2003 Revision. This regulation incorporates the provisions governing the processing, packaging, and sale of Grade A milk and milk products, including yogurt, fermented milk products, whey, whey products, condensed, and dry milk products. The 25th revision of the *Grade “A” PMO* incorporates new knowledge into public health practices.

The USPHS did not produce the *Grade “A” PMO* alone. As with preceding editions, it was developed with the assistance of Milk Regulatory and Rating Agencies at every level of federal, state, and local government. All segments of the dairy industry,

including health and agriculture regulators, producers, milk-plant operators, equipment manufacturers, associations, and educational and research institutions assisted in producing the *Grade “A” PMO*.

The USPHS recommended *Grade “A” PMO* is the basic standard used in the voluntary Cooperative State-USPHS Program for the National Conference on Interstate Milk Shipments (NCIMS); a program participated in by all 50 states, the District of Columbia and U.S. Trust Territories. The NCIMS, in accordance with the “Memorandum of Understanding” with the FDA, recommends changes and modification to the *Grade “A” PMO* at its biennial conferences.

The *Grade “A” PMO* is incorporated by reference in federal specification for procurement of milk and milk products. It is used as the sanitary regulation for milk and milk product served on interstate carriers and is recognized by the public health agencies, the milk industry, and many others as the national standard for milk sanitation.

The USPHS has legal jurisdiction for the enforcement of milk sanitation standards, in the case of interstate commerce. It also serves in an advisory and simulative capacity and is designed primarily to assist state and local regulatory agencies. The milk safety program aims to promote the establishment of effective and well-balanced milk sanitation programs in each state; to stimulate the adoption of adequate and uniform state and local milk control legislation; and to encourage the application of uniform enforcement procedures through appropriate legal and educational measures. Its enforcement becomes a function of the local or state authorities when the *Ordinance* is adopted locally.

INSPECTION OF MILK SAFETY

State and local regulatory agencies are responsible for the enforcement of sanitation requirements on dairy farms, milk hauling receiving and transfer stations, and in processing plants. The FDA’s primary function under the Federal/State Milk Safety Cooperative Program is to provide technical assistance to the states in the implementation and enforcement of their regulations. This assistance is provided through district and regional milk specialists and the Center for Food Safety and Applied Nutrition’s (CFSAN) Milk Safety Team. The inspection program is carried out by the state regulatory agency under the requirements of the Cooperative Program on the NCIMS. As a result, there is a greater degree of reciprocity between

sates on acceptance of inspections. The NCIMS *Procedures* document contains information for establishing milk sanitation standards, rating procedures, sampling procedures, laboratory evaluation, and sample collector procedures. (USDHHS Procedures, 2005)

The *Procedures* requires that producer farms and processing facilities are inspected by the state regulatory agencies on a routine basis at a minimum of twice a year with many state regulatory agencies inspecting on a four per year schedule. These farms and dairy plants are also inspected under the Interstate Milk Shipper (IMS) program to determine the “rating” of all plants electing to participate in the IMS program. State or local ratings must be conducted by a certified USPHS representative. These ratings are conducted no less than once every 24 months (but no more than 15 days) and are based on compliance with the *Grade “A” PMO* requirements. The ratings provide an assessment of state and local sanitarians activities regarding public health control and milk quality control. Rating inspections are expressed in terms of percentage compliance. For example, if the milk plant and dairy farms comply with all requirements of the *Grade “A” PMO*, the Sanitation Compliance Rating of the pasteurized milk supply would be 100%. However, if the plant of some of the dairy farms fails to satisfy one or more of these requirements the Sanitation Compliance Rating would be reduced in proportion to the amount of milk and milk products involved in the violation, and to the relative public health significance of the violated item(s). Additionally, the USPHS is obligated to conduct periodic “check ratings” to assure validity and uniformity with each state’s ratings. (USDHHS Methods, 2003)

Farm Requirements

The *Grade “A” PMO* sections on raw milk established the requirements and standards for milk production and farm conditions. This requires that milking animals are disease-free and do not show signs of secretion of abnormal milk such as blood or mastitis. Milk from cows that have been treated with medications or antibiotics must be properly separated. The milking barn, stable, or parlor must be properly constructed with floor that are concrete or impervious to easily maintain cleanliness. Walls and ceilings should be smooth, painted, or finished making it dust-tight in order to reduce the likelihood of dust and extraneous material from getting into the milk. There must be sufficient light during the day and night, as

well as good air circulation to prevent condensation and excess odors.

Milking barns must be kept clean and the cow yard should be graded for proper drainage to prevent standing water or excess accumulation of organic waste. The milk house should be of sufficient size and provide proper cooling, handling, and storage of milk. It should include proper facilities to wash, sanitize, and store of milk container and utensils. Milk houses must have tight-fitting doors to the milking barn; water that is piped under pressure with an adequate supply of hot water. The milk cooling must be monitored by accurate accessible temperature recording devices installed in the milk line and milk must be cooled to below 7°C (45°F). The milk house must be kept clean to reduce the likelihood of contamination of the milk. Every dairy farm should have at least one conveniently located toilet. Water for the milk house must be from a supply properly located and protected to provide safe and sanitary water quality.

Milking equipment and utensils for handling and storage of milk on the farm must be made of smooth, nonabsorbent, corrosion-resistant, and nontoxic material and constructed in such a way that they can be easily cleaned. Multiuse woven material is not allowed for straining milk. Strainers, if used, must be a perforated design or constructed to utilize a single-use strainer media such as paper or cloth. Details for plans of mechanically cleaned milk pipeline systems must be submitted to the state regulatory agency for written approval prior to installation.

Utensils and equipment used for milk handling, storage, or transportation must be cleaned after each use, sanitized before reusing, and stored to assure complete drainage and protection from contamination. Additionally, effective measures must be in place to prevent contamination by insects, rodents, and the chemicals used to control these pests.

Milking must be done in the milking barn, stable, or parlor. The cow's flanks, udder, bellies, and tails must be free from visible dirt. Udders and teats should be cleaned and dried before milking. Teats should be treated with a sanitizing solution just prior to the time of milking and dried before milking. However, alternative udder preparation methods may be allowed once they are evaluated and approved by the FDA.

Milking house operations, equipment, and facilities should be conducted to prevent any contamination of milk, equipment, container, or utensils. Vehicles used to transport the milk from the dairy farm

to the milk plant, receiving station, or transfer station should be constructed to protect the milk from sunlight, freezing, and contamination. Cleaners and sanitizers used on the farm should be properly identified. Animal drugs should be properly labeled and segregated for their use on nonlactating animals. Unapproved drugs should not be used. Personal cleanliness is important, therefore hand-washing facilities must be provided.

Additionally, the dairy farm is responsible for assuring that the raw milk for pasteurization is be cooled to 10°C (50°F) or less within 4 hours or less of the commencement of the first milking, and to 7°C (45°F) or less within 2 hours after the completion of milking provided that the blend temperature after the first milking and subsequent milking does not exceed 10°C (50°F).

Milk Transportation

The sanitary requirements for transportation of raw milk from the farms to processing plant are also detailed in the *Grade "A" PMO*. These policies may be found under the sections on raw milk and regulations pertaining to raw milk receiving stations and transfer stations.

Milk is collected and stored at the farms in a cooled bulk tank, then picked up daily or every other day by bulk milk transportation trucks. These trucks must be made of smooth, nonabsorbent, corrosion-resistant, nontoxic, material that can be easily cleaned, and constructed to protect the milk from dust or airborne contamination.

The bulk milk hauler is often responsible for collecting official milk samples as well as transporting raw milk from a farm to a receiving station, transfer station, or a milk processing plant. The bulk milk hauler is required to have training and pass an examination with a score of 70% or greater related to sanitation, sampling, and weighing procedures, including proper use and cleaning of equipment, and record-keeping requirements. The bulk milk hauler is issued a permit upon successful completion of the examination. The state regulatory agency must observe the bulk milk hauler's techniques at one or more farms every 24 months for the permit to remain valid.

Bulk milk tank trucks are also permitted and inspected by the state regulatory agency annually. If construction or repair defects are noted, the milk tank truck should be removed for service until repairs and sufficient cleaning are verified. The milk tank truck

standards encompass the following areas: properly constructed equipment to hold milk at correct temperatures of 7°C (45°F) or less, adequate milk sampling equipment, and a tag affixed to the truck's outlet valve to verify washing and sanitizing records. When the bulk milk haulers are responsible for obtaining and transporting milk samples for official laboratory analysis, they must complete records verifying the chain-of-custody for the samples.

Bulk raw milk from farms may be transported directly to the milk-processing plants or it may be held at a transfer station where it is pooled with other raw bulk milk loads. The transfer station unloads smaller bulk milk trucks into holding silos and then reloads the commingled raw milk into larger over-the-road tanker trucks for delivery to processing plants. All vehicles and milk tank trucks containing milk or milk products should be legibly marked with the name and address of the milk plant or hauler in possession of the contents.

Milk tank trucks transporting raw, heat-treated, or pasteurized milk and milk products to a milk plant from another milk plant, receiving station, or transfer station are required to be marked with the name and address of the milk plant or hauler and should be sealed. Additionally, a statement should be prepared for each shipment containing at least the following information:

1. Shipper's name, address, and permit number. Each milk tank truck containing milk should include the IMS Bulk Tank Unit (BTU) identification number(s) or the IMS Listed Milk Plant Number, for farm groups listed with a milk plant, on the weight ticket or manifest.
2. Permit identification of hauler, if not an employee of the shipper.
3. Point of origin of shipment.
4. Tanker identification number.
5. Name of product.
6. Weight of product.
7. Temperature of product when loaded.
8. Date of shipment.
9. Name of supervising Regulatory Agency at the point of origin of shipment.
10. Whether the contents are raw or pasteurized, or in the case of cream, low fat, or skim milk, whether it has been heat-treated.
11. Seal number on inlet, outlet, wash connections, and vents.
12. Grade of product.

Processing Plant

Manufacturing plants that process milk products, cultured milk product such as sour cream, yogurt, and fermented milk products are subject to the food safety requirements in the *Grade "A" PMO* section on Standards for Grade "A" Pasteurized, ultra-pasteurized, and aseptically processed milk and milk products. These requirements dictate the construction of floors, walls, ceilings, door, and windows as well as proper lighting and ventilation. Floors in all rooms of the processing facility where milk products are handled, processed, and sorted or in which milk container, utensils, and equipment are washed must be constructed of concrete or other equally impervious and easily cleanable material. The floor must be properly sloped with trapped drains. Storage rooms for dry ingredients need not have drains and may have floor constructed of wood. Walls and ceilings should be smooth, light-colored, washable, and in good repair. Doors and windows should prevent access to insects and rodents and all opening to the outside must have solid doors or glazed windows. However, other methods of effectively protecting opening to the outer air such as screening, fans, air curtains, and properly constructed flap may be used provided that the entrance of insect and rodents are prevented.

The processing plant must be designed so the separate rooms are provided for each of the following operations:

1. The pasteurizing, processing, cooling, reconstitution, condensing, drying, and packaging of milk and milk products.
2. Packaging of dry milk or milk products.
3. The cleaning of milk cans, containers, bottles, cases, and dry milk or milk product containers.
4. The fabrication of containers and closures for milk and milk products.
5. Cleaning and sanitizing facilities for milk tank trucks in milk plants receiving milk or whey.
6. Receiving cans of milk and milk products in milk plants receiving such cans.

Every milk processing plant should have toilet and hand-washing facilities with hot and cold running water, soap, and individual sanitary towels or approved hand-drying devices. The water supply should be adequate, safe, and of sanitary quality. The water supply may be approved as safe from the State Water Control Authority, or in the case of individual water systems (wells), comply with construction specifications and bacteriological standards.

The processing facility should be kept clean, neat, and free of evidence of insects and rodents in order to reduce the likelihood of contamination of the milk or milk products. All piping, floors, walls, ceilings, fans, shelves, tables, and nonproduct contact surfaces should be cleaned. Trash and solid waste must be kept in covered containers.

All sanitary piping, fittings, connections, multiuse containers, and equipment which come in contact with milk and milk products should be smooth, impervious, corrosion-resistant, nontoxic, and easily cleanable material which is approved for food contact surfaces. All sanitary piping, connections, and fitting must meet the following requirements:

- a. Stainless steel of the AISI 300 series
- b. Equally corrosion-resistant metal which is nontoxic and nonabsorbent
- c. Heat-resistant glass
- d. Plastic or rubber and rubber-like materials which are relatively inert, resistant to scratching, scoring, decomposition, crazing, chipping, and distortion under normal use conditions; must be nontoxic, fat resistant, relatively nonabsorbent, not impart flavor or odor to the milk or milk products, and maintain their original properties under repeated use conditions
- e. Designed to permit easy cleaning, maintained in good repair, free of breaks or corrosion, and contain no dead ends of piping in which milk or milk product may collect.

Equipment, containers, and utensils should have joints that are flush and smooth finish. All openings to tanks, vats, and separators are protected by raised edges to prevent the entrance of surface drainage and condensation-diverting aprons should be provided. There must not be threaded fitting in milk contact areas. Strainers, if used, should be a perforated design or constructed to utilize a single-use strainer media, such as cloth or paper. Woven material may be used only where it is impractical to use perforated strainers. However, woven strainers must be thoroughly mechanically cleaned.

All single-service containers, closures, gaskets, and other articles that contact milk should be nontoxic and should be manufactured, packaged, transported, and handled in a sanitary manner and may not be reused.

One of the most critical food safety procedures is proper cleaning and sanitation of containers and equipment that are used for processing, culturing,

filling, packaging, and storage of milk and fermented milk products. All multiuse containers and utensils such as tanks, lines, vessels, pasteurizers, and filling equipment must be cleaned at least once a day. Storage tanks should be cleaned when emptied at least every 72 hours and records must be readily available to verify storage times. Cleaning frequencies beyond these requirements are allowed after review and acceptance of specific information by the state regulatory agency in consultation with FDA. Pipelines and equipment designed for mechanical cleaning (cleaning-in-place, CIP) must meet specific requirements of being equipped with a temperature-recording device that provides a continuous record of the time and temperature, cleaning solution velocity, and the presence, strength, or cleaning solution chemicals. For manual washing, there must be a two-compartment wash-and-rinse vat. After cleaning, milk product containers, utensils, and equipments should be stored to assure complete drainage and protection from contamination.

Single-service caps, cap stock, containers, gaskets, and other articles for use in direct contact with milk and milk products must be stored in sanitary wrapping or cartons and kept in a clean, dry place until used. This includes the containers and lids used for yogurt and fermented milk packages.

Throughout the milk processing plant, ingredients in process product, packaging and finished products must be protected from contamination. This includes discarding spilled, overflowed, or leaked milk and milk products. All poisonous or toxic materials should be properly labeled and stored in a separate area and used to preclude contamination. All product contact surfaces must be covered or otherwise protected to prevent the exposure to insect, dust, condensation, and other contamination. Many openings, including valves, piping attached to milk storage, milk tank trucks, and pumps vats should be capped or properly protected. Air must be free of oil, rust, rust-recessive moisture, extraneous materials, and odor when air pressure is used for agitation or the movement of milk. The use of steam in contact with milk requires it to be of culinary quality.

During processing, pipelines and equipment used to contain or conduct milk and milk products should be effectively separated from tanks or circuits containing cleaning and/or sanitizing solutions. This can be accomplished by physically disconnecting all connection points, by separation with two automatically controlled valves or by a single-bodied double seat

Table 7.1. Pasteurization Temperature versus Time

Temperature	Time
63°C (145°F) ^a	30 minutes
72°C (161°F) ^a	15 seconds
89°C (191°F)	1.0 second
90°C (194°F)	0.5 seconds
94°C (201°F)	0.1 seconds
96°C (204°F)	0.05 seconds
100°C (212°F)	0.01 seconds

^a If the fat content of the milk product is 10% or more, or if it contains added sweeteners, or is concentrated (condensed), the specific temperature shall be increased by 3°C (5°F).

valve with a drainable opening between tanks and circuits containing cleaning and/or sanitizing solutions from pipelines and equipment used for milk or milk products. Additionally, there should be no physical connection between water, nondairy products, unpasteurized dairy product, and pasteurized milk and milk products.

Pasteurization is the only practical, commercial measure that, if properly applied to all milk, will destroy all milk-borne disease organisms. It has been demonstrated that the time–temperature combinations specified by this *Grade “A” PMO*, if applied to every particle of milk or milk product, will kill all milk-borne pathogens. Although pasteurization destroys the organisms, it does not destroy the toxins that may be formed in milk and milk products when certain staphylococci bacteria are present. Staphylococcal toxin can result from udder infections when the milk or milk products are not properly refrigerated before pasteurization. Such toxins may cause severe illness. The requirements and equipment specifications for milk pasteurization are given in Table 7.1.

Table 7.2 shows the time–temperature combinations for pasteurization of eggnog.

Detailed information about the design, installation, and operation of the milk pasteurizing equipment is also dictated by the *Grade “A” PMO*. The oversee-

ing regulatory agency performs specific tests on the pasteurizer’s critical instruments and devices upon initial installation and at least once every 3 months, and then applies seals to specific equipment that regulate the temperature or flow rate. All temperature and flow rate pasteurization records are required to be preserved for a period of 3 years.

Maintaining milk at proper temperatures to avoid bacterial growth and spoilage is critical to product quality and safety. All raw milk and milk products should be maintained at 7°C (45°F) or less until processed. All pasteurized milk and milk products, except those to be cultured, should be cooled immediately prior to filling or packaging in an approved equipment, at a temperature of 7°C (45°F). This exemption for higher temperature during culturing has also been applied to fermentation that occurs in the final package, such as cup-set yogurt. All pasteurized milk and milk products should be stored at a temperature of 7°C (45°F) or less until further processed. To verify proper refrigeration, every refrigerated room or tank in which milk or milk products are stored should be equipped with an accurate indicating thermometer. On delivery vehicles, the temperature of milk and milk products should not exceed 7°C (45°F). However, aseptically processed milk and milk products to be packaged in hermetically sealed containers are exempt from these cooling requirements.

Filling, packaging, and capping of pasteurized milk products must be done at the place of pasteurization in a sanitary manner by approved mechanical equipment. The packaging equipment and supply lines must be equipped with covers to prevent contamination from reaching the inside of the filler bowl and drip deflector designed to divert condensation away from open containers. Container in-feed conveyors to automatic bottling or packaging machines should have overhead shields to protect the bottles or packaging from contamination. Caps and closures must be applied in a manner where they cannot be removed without detection help to provide assurance to the consumer that the milk and milk products have not been contaminated after packaging. All packaging must be handled in a sanitary manner.

Employees working in the milk processing plant must maintain a high degree of personal cleanliness. Hands must be thoroughly washed before commencing plant functions or resuming work after visiting the toilet, eating, or smoking. Employees must wear clean outer garments and adequate hair coverings. Persons affected with any disease capable of being

Table 7.2. Eggnog Pasteurization Temperature versus Time

Temperature	Time
69°C (155°F)	30 minutes
80°C (175°F)	25 seconds
83°C (180°F)	15 seconds

transmitted to others through the contamination of food should not work at a milk plant in any capacity which brings them into direct contact with pasteurized milk or aseptically processed milk or milk product-contact surfaces.

All vehicles used to transport pasteurized milk and milk products should be constructed and operated so that the milk and milk products are maintained at 7°C (45°F) or less and are protected from contamination. Milk tank cars, milk tank trucks, and portable shipping bins should not be used to transport or contain any substances that may be toxic or harmful to humans.

The surroundings of a milk plant should be kept neat and clean to prevent attracting rodents, flies and other insects which may contaminate the milk or milk products. Insecticides and rodenticides must be approved for use in milk plants and used in accordance with label recommendations.

HACCP

For detailed discussion of HACCP and food safety measures, the reader is referred to Chapter 22.

History of HACCP

The use of the hazard analysis and critical control point (HACCP) system is not new to the dairy industry. HACCP is a logical, simple, effective, and highly structured system of food safety control. The HACCP system was introduced to the food industry as a spin-off of the space program during the 1960s. The National Aeronautics and Space Administration (NASA) used HACCP to provide assurance of the highest quality available for components of space vehicles. This program, to develop assurance of product reliability, was carried over into the development of foods for astronauts.

Background

HACCP is a management tool that provides a structured and scientific approach to the control of identifiable hazards. HACCP is a logical basis for better decision-making with respect to product safety. HACCP is internationally recognized as an effective means of controlling food safety hazards and is endorsed as such by the joint Food and Agriculture Organization (FAO) of the World Health Organization Codex Alimentarius Commission. The U.S. National Advisory Committee on Microbiological Criteria for

Foods (NACMCF) has also endorsed it. The HACCP concept enables those operating and regulating under an HACCP plan to move to a preventive approach, whereby potential hazards are identified and controlled in the manufacturing environment, that is, prevention of product failure. HACCP allows for a preventive systematic approach to food safety.

Voluntary Participation

The NCIMS HACCP program is a voluntary alternative to the traditional inspection system. Milk plant, receiving station, or transfer station can participate in the voluntary NCIMS HACCP program only when the state regulatory agency responsible for the oversight of the facility agrees to participate with the dairy plant(s), receiving station(s), and transfer station(s) in the NCIMS HACCP program. Management responsible for both the state and dairy plant, receiving station, or transfer station must be willing to provide the resources needed to develop and implement a successful HACCP system. Both parties must provide written commitment to each other that the necessary resources to support participation in the NCIMS HACCP program will be made available.

HACCP Principles

Following are the seven HACCP principles to be included in a HACCP plan:

1. Conduct a hazard analysis
2. Determine the critical control points
3. Establish critical limits
4. Establish monitoring procedures
5. Establish corrective actions
6. Establish verification procedures
7. Establish record-keeping and documentation procedures

Prerequisite Programs Prior to the implementation of an HACCP plan, there is a requirement for dairy plants, receiving stations, and transfer stations to develop, document, and implement written prerequisite programs (PPs). PPs provide the basic environment and operating conditions that are necessary for the production of safe, wholesome food. Many of the conditions and practices are specified in federal and state regulations and guidelines.

The seven principles of HACCP are also called the HACCP plan. When combined with the PPs, they constitute an HACCP system. The NCIMS HACCP program combines the HACCP system and other

prescribed *Grade “A” PMO* criteria, such as drug residue testing and trace back, use of milk only from supplies that have been awarded a milk sanitation compliance rating of at least 90% or from an acceptable IMS HACCP listed source and labeling requirements. When properly implemented, the HACCP program will provide assurance of milk and milk product safety that is equivalent to that provided under the traditional inspection system.

STANDARDS AND REGULATIONS

Standards for Containers and Closures

Single-service containers and closures, such as plastic jugs, plastic-coated paperboard milk containers, plastic tubs, lids, and aluminum aerosol cans are used by the dairy industry for packing milk and milk products. Industry applied quality assurance controls for manufacturing and handling of the materials have made it possible for these products to reach the point of use in a sanitary condition free from toxic materials which may migrate into milk or milk products. Standards set forth in the *Grade “A” PMO*, Appendix J, insure the production of sanitary containers and closures for milk and milk products. The standards include the bacterial requirements, fabrication plant, equipment, and processing and packaging standards as well as materials, waxes, adhesives, sealants, and inks that can be used. Approval of certified single-service containers and closures plants is published in the *IMS List* quarterly.

Labeling

Labeling of bottles, containers, and packages containing milk or milk products are defined in applicable requirements of the Federal Food Drug and Cosmetic Act (FFDCA), the *Nutrition Labeling and Education Act* (NLEA) of 1990 and regulations developed there under the Federal Code of Regulations Title 21. More detailed information on FDA-labeling regulations can be found in Chapter 6. However, in addition to federal requirements, the *Grade “A” PMO* requires additional labeling as follows.

All bottles, containers, and packages containing milk or milk products except milk tank trucks, storage tanks, and cans of raw milk from individual dairy farms should be conspicuously marked with the following:

1. The identity of the milk plant where pasteurized, ultra-pasteurized, aseptically processed, condensed, or dried. This may be accomplished by stating the company name and location listing the city and state by printing on the container a unique identification number assigned by the state to each plant which is the “IMS Listed Milk Plant Number.”
2. The words “keep refrigerated after opening” in the case of aseptically processed milk and milk products.
3. The common name of the hooved mammal producing the milk should precede the name of the milk or milk product when the product is made from milk other than cow’s milk. As an example, “Goat,” “Sheep,” “Water Buffalo,” or “Other Hooved Mammal” milk or milk products.
4. The word “Grade ‘A’” on the exterior surface. Acceptable locations should include the principal display panel, the secondary or informational panel, or the cap/cover. The term “Grade A” may not solely appear in the ingredient statement.
5. The word “reconstituted” or “recombined,” if the product is made by milk subject to reconstitution, recombined milk or milk ingredients.

All labeling terms must be truthful and not misleading as dictated by the FFDCA. Grade designations, such as “Grade ‘AA’ Pasteurized,” “Selected Grade ‘A’ Pasteurized,” “Special Grade ‘A’ Pasteurized,” etc., give the consumer the impression that such a grade is significantly safer than Grade “A.” Such an implication is false because the *Ordinance* requirements for Grade A pasteurized, ultra-pasteurized, or aseptically processed milk when properly enforced will ensure that this grade of milk will be as safe as milk can practically be made. Descriptive labeling terms must not be false and misleading and may not be used in conjunction with the Grade “A” designation or name of the milk or milk product. If descriptive terms are used in conjunction with attributes of the product other than milk safety, i.e., “special select strawberries” for strawberry yogurt or “rich cream texture,” these labeling terms should not be in a location immediately preceding or following the name of the food. Creating physical distance and employing graphic enhancements such as distinctive type styles, bursts, and other techniques generally are effective ways of distinguishing optional information from the required information. (USDHHS PMO, 2003).

EXAMINATION OF MILK PRODUCTS

Table 7.3 gives the standards for Grade A dairy products.

1. The identity of the milk plant where pasteurized, ultra-pasteurized, aseptically processed,

Table 7.3. Chemical, Physical, Bacteriological, and Temperature Standards

Grade "A" raw milk and milk products for pasteurization, ultra-pasteurization, or aseptic processing	Temperature	Cooled to 10° C (50° F) or less within 4 hours or less, of the commencement of the first milking, and to 7° C (45° F) or less within 2 hours after the completion of milking. Provided, that the blend temperature after the first milking and subsequent milkings does not exceed 10° C (50° F). Individual producer milk not to exceed 100,000 per mL prior to commingling with other producer milk.
	Bacterial limits	
	Drugs	No positive results on drug residue detection methods as referenced in Section 6—Laboratory Techniques
	Somatic cell count ^a	Individual producer milk not to exceed 750,000 per mL.
Grade "A" pasteurized milk and milk products and bulk shipped heat-treated milk products	Temperature	Cooled to 7° C (45° F) or less and maintained thereafter.
	Bacterial limits ^b Coliform ^d	20,000 per mL, or gram. ^c Not to exceed 10 per mL. Provided that in the case of bulk milk transport, tank shipments shall not exceed 100 per mL.
	Phosphatase ^e	Less than 350 milliunits/liter for fluid products and other milk products by the Fluorometer or Charm ALP or equivalent.
	Drugs ^b	No positive results on drug residue detection methods as referenced in Section 6—Laboratory Techniques which have been found to be acceptable for use with pasteurized and heat-treated milk and milk products.
Grade "A" pasteurized concentrated (condensed) milk and milk products	Temperature	Cooled to 7° C (45° F) or less and maintained thereafter unless drying is commenced immediately after condensing.
	Coliform	Not to exceed 10 per gram. <i>Provided</i> that in the case of bulk milk transport, tank shipments shall not exceed 100 per mL.

Table 7.3. (cont.)

Grade "A" aseptically processed milk and milk products	Temperature	None.
	Bacterial limits	Refer to 21 CFR 113.3(e)(1) ^f
	Drugs ^b	No positive results on drug residue detection methods as referenced in Section 6—Laboratory Techniques that have been found to be acceptable for use with aseptically processed milk and milk products.
Grade "A" Nonfat dry milk	Butterfat	Not more than:
	Moisture	1.25%
	Titratable acidity	4.00%
	Solubility index	0.15%
	Bacterial estimate	1.25 mL.
	Coliform	30,000 per gram
	Scorched particles disc B	10 per gram
	Temperature	15.0 per gram
Grade "A" whey for condensing		Maintained at a temperature of 45°F (7°C) or less, or 63°C (145°F) or greater, except for acid-type whey with a titratable acidity of 0.40% or above, or a pH of 4.6 or below.
Grade "A" pasteurized condensed whey and whey products	Temperature	Cooled to 7°C (45°F) or less during crystallization, within 48 hours of condensing.
Grade "A" dry whey, Grade "A" dry whey products, Grade "A" dry buttermilk, and Grade "A" dry buttermilk products	Coliform Limit	Not to exceed 10 per gram
	Coliform limit	Not to exceed 10 per gram

^a Goat milk 1,000,000 per mL.

^b Not applicable to acidified or cultured products.

^c Results of the analysis of dairy products which are weighed in order to be analyzed will be reported in # per gram. (Refer to the current edition of the SMEDP.)

^d Not applicable to bulk shipped heat-treated milk products.

^e Not applicable to bulk shipped heat-treated milk products; UP products that have been thermally processed at or above 138°C (280°F) for at least 2 seconds to produce a product which has an extended shelf life (ESL) under refrigerated conditions; and condensed products.

^f 21 CFR 113.3(e)(1) contains the definition of "Commercial sterility."

Source: USDDH SPMO, 2003

In order to verify the quality and safety of the milk and milk products to the *Grade "A" PMO*, it is required that raw milk, commingled milk in the silos intended for processing, and finished products be sampled and tested by state regulatory agencies at a specific frequency. The products must meet chemical, bacteriological, and temperature standards. It is required that the state regulatory agency collect and test official samples at least four samples during any consecutive 6 months. However, many state regulatory agencies sample and test monthly. The samples must include each fat level and both plain and flavored products for finished milk and milk products. Therefore, if a plant produces plain low-fat yogurt, flavored low-fat yogurt, and flavored nonfat yogurt, all three products must be sampled. It is not necessary to sample each flavor monthly, but usually different flavors are chosen each time the product is sampled.

Testing of official samples must be done in laboratories that are certified under the IMS program and by technicians that have been certified to perform the specific required tests. Requirements for laboratories are governed by the *Evaluation of Milk Laboratories* (EML). Sampling procedures and required laboratory tests must be in compliance with the most current edition of *Standard Methods for the Examination of Dairy Products* (SMEDP) of the American Public Health Association and the most current edition of *Official Methods of Analysis of AOAC INTERNATIONAL* (OMA). Chapter 23 of this book discusses various analytical procedures used for testing dairy products.

Imports

The United States is a signatory of the World Trade Organization (WTO) agreement, which allows countries to establish measures to insure safety of food within their countries. The measures, however, must be applied in a manner so that they do not arbitrarily discriminate between products from different countries or treat domestic products more favorably than imported products without justification. The determination of equivalence is made by the importing country based on whether the exporting country's measures meet the level of protection deemed appropriate by the importing country as provided by its own measures.

The FDA and the NCIMS have identified and mutually accepted three options which are consistent with NCIMS "Procedures" and allow states to receive

PMO define "Grade A" products produced outside of the United States.

These options are as follows:

1. A dairy firm outside of the United States could contract with any current NCIMS member's regulatory agency to provide the "Grade A" milk safety program in total. This would include the regulatory licensing, dairy farm and milk plant inspection and sampling, pasteurization equipment tests, laboratory certification and rating/NCIMS listing certification. To use this option, the firm would be required to abide by all applicable NCIMS regulatory and rating requirements and the regulatory/rating agency would have to agree to treat the firm as if it were located within its jurisdiction for all purposes including inspection and enforcement. Ratings of the firm would be check-rated by the FDA.
2. The importing country may become a full member of the NCIMS subject to all NCIMS rules and enjoying all privileges of a U.S. state. This would require, among other things, that the milk regulatory agencies of the importing countries adopt and enforce rules and regulations that are the same as those required in the United States and abide by all applicable NCIMS regulatory and rating requirements. Their ratings would be check-rated by FDA in the same way as state ratings. The FDA would certify their rating, sampling surveillance, and laboratory evaluation officers.
3. The FDA can evaluate the importing country's system of assuring the safety of dairy products and compare the effect of that system with the effect of the U.S. system on the safety of dairy products produced domestically. The NCIMS has adopted a procedure to accept FDA finding of equivalence and to allow NCIMS member states to accept products produced within the scope of such a finding.

Additionally, Grade "A" milk products have restrictions in the use of imported dairy ingredients. As specified in the *Grade "A" PMO*, Grade "A" dairy products must use only Grade "A" dairy ingredients except that small amounts of functional ingredients (total of all such ingredients should not exceed 5% by weight of the finished blend) which are not Grade "A" are allowed in Grade "A" when the finished ingredient is not available in Grade "A" form, that is, sodium caseinate (USDHHS PMO, 2003).

EQUIPMENT STANDARDS

The specific requirements for equipment for milking, milk transportation, storage, and processing are explained in the *Grade “A” PMO*. To comply with the sanitary design and construction standards of the PMO, equipment manufactured in conformity with 3-A Sanitary Standards must be evaluated by the state regulatory agency prior to installation. 3-A Sanitary Standards for dairy equipment are promulgated jointly by the Sanitary Standards Subcommittee of the Dairy Industry Committee, the Committee on Sanitary Procedure of the International Association for Food Protection (IAFP), and the FDA Milk Safety Branch.

3-A Sanitary Standards Symbol

The 3-A symbol was introduced in 1927 and is used to identify equipment that meets 3-A Sanitary Standards for design and fabrication. Use of the 3-A symbol is governed by 3-A Sanitary Standards, Inc. (3-A SSI).

Once a 3-A Sanitary Standard has been developed and becomes effective, manufacturers may receive authorization from the 3-A Symbol Council to use the symbol. Voluntary use of the 3-A symbol on dairy and food equipment serves three important purposes:

1. Assures processors that equipment meets sanitary standards;
2. Provides accepted criteria to equipment manufacturers for sanitary design; and
3. Establishes guidelines for uniform evaluation and compliance by Sanitarians.

3-A SSI formulates standards and practices for the sanitary design, fabrication, installation, and cleanliness of dairy and food equipment or systems used to handle, process, and package consumable products where a high degree of sanitation is required. These standards and practices are developed through the cooperative efforts of industry experts. Its ultimate goal is to protect consumable products from contamination and to insure that all product surfaces can be mechanically cleaned-in-place (CIP) or easily dismantled for manual cleaning.

3-A Accepted Practices cover a system, which is defined as a set of connected equipment and machinery that forms as a whole or works together. In addition to the criteria for equipment, a practice may also provide specification for sanitary installation and legal controls.

3-A Sanitary Standards provide material specifications, design criteria, and other necessary information for equipment types to satisfy public health concerns. 3-A Standards are available for more than 70 equipment types, from fittings centrifugal pumps, heat exchangers, valves, membranes, and CIP spray devices to silo tanks. 3-A criteria are universally accepted by equipment manufacturers, fabricators, users, and sanitarians. The 3-A symbol, where authorized by 3-A SSI, is used by equipment manufacturers and fabricators to indicate conformance to 3-A standards.

In order for dairy and food equipment manufacturers to use the 3-A symbol, they must file an application with the 3-A Symbol Council office signifying that the equipment is compliant with all provisions of that standard. A statement of quality controls in place must be submitted along with drawings or pictures of the equipment. The Council may also request additional materials to insure compliance on complex subassemblies. The Council reviews the application and, if all areas are in compliance under that specific 3-A standard, the manufacturer is permitted to use the 3-A symbol.

Equipment manufacturers are required to place the serial number of the 3-A standard with which it complies adjacent with the 3-A symbol on their equipment.

A listing of authorized holders of 3-A symbol certification can be found on the 3-A Sanitary Standards website. The listing is organized by standards for each type of equipment and provides the manufacturing company's information and if relevant, the model number of the piece of equipment that has received authorization.

MILK PRICING—U.S. FEDERAL MILK MARKETING ORDERS

Background of Federal Orders

The Federal Milk Marketing Orders system is a regulatory function administered by the United States Department of Agriculture (USDA). The Federal Orders have evolved significantly since their first legislative introduction in 1937. The objective of the Federal Orders is to stabilize markets by placing certain requirements on the pricing and handling of milk in the area it covers, and ultimately, assures that an adequate supply of wholesome milk is available and will continue to be available at a reasonable price to consumers. There are 11 regions in the United States that are regulated by a Federal Order. (Fig. 7.4) Regions

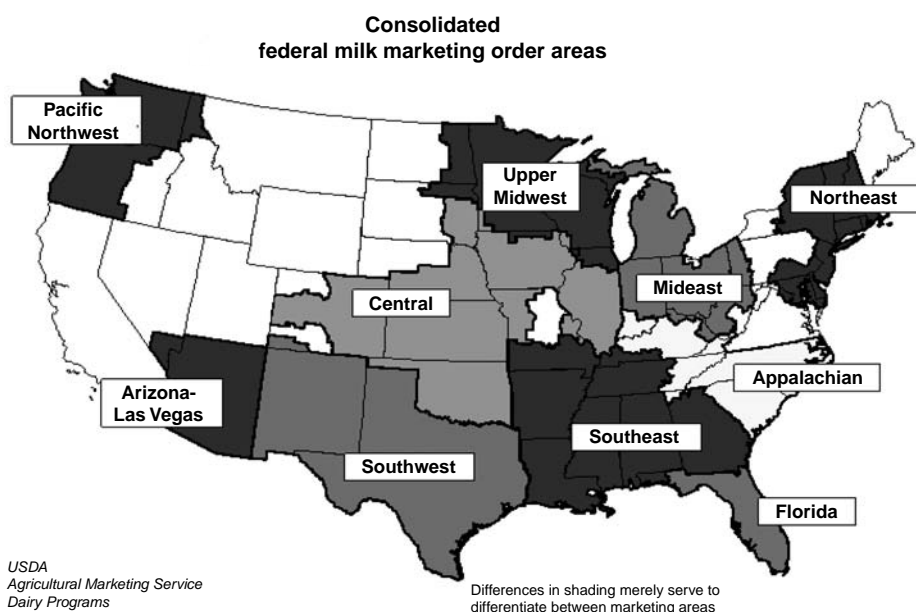


Figure 7.4. Consolidated federal milk orders areas.

that are not subject to a Federal Order have a state milk marketing order such as in California where the pricing system is akin to the Federal Orders, or they may be unregulated (USDA, 2006).

The Federal Milk Marketing Orders are concerned primarily with orderly marketing of raw Grade “A” milk from producer to processor. Classified pricing and pooling are the two key elements for the Federal Milk Orders which set minimum prices for more than 70% of the Grade A milk produced in the United States. A major function of the Federal Orders is computing minimum prices for raw Grade A milk that handlers must pay to dairy farmers. The Federal Milk Marketing Orders system has been developed to pool the proceeds of all qualified milk sales (regardless of the actual end use of an individual producer’s milk) in order to insure that all producers in an area receive a uniform price for their milk—regardless of how their milk was used.

Classified Pricing

The Federal Milk Marketing Orders program uses product price formulas to determine milk component values that are combined to calculate monthly class prices. The factors in the formulas are dairy product

prices, which change monthly, and make allowances and product yields, which are set in the formulas. The dairy product prices are those collected by USDA from weekly surveys of dairy product manufacturers that sell specific products on a bulk, wholesale basis (Jesse and Cropp, 2004).

Federal orders define the following four classes of milk, from highest to lowest value (under most circumstances):

1. Class I is milk used for beverage products. This includes “white” whole, low-fat, and skim milk in all container sizes, chocolate and other flavored milks, liquid buttermilk, and eggnog.
2. Class II is milk used for soft manufactured products like yogurt and cultured dairy products, sour cream, ice cream, and other frozen dairy desserts, cottage cheese, and creams.
3. Class III is milk used to manufacture cream cheese and hard cheeses.
4. Class IV is milk used to make butter and dry milk products—principally nonfat dry milk.

Producer Prices

The Federal Orders require milk handlers in a marketing area to pay dairy farmers (producers) no less

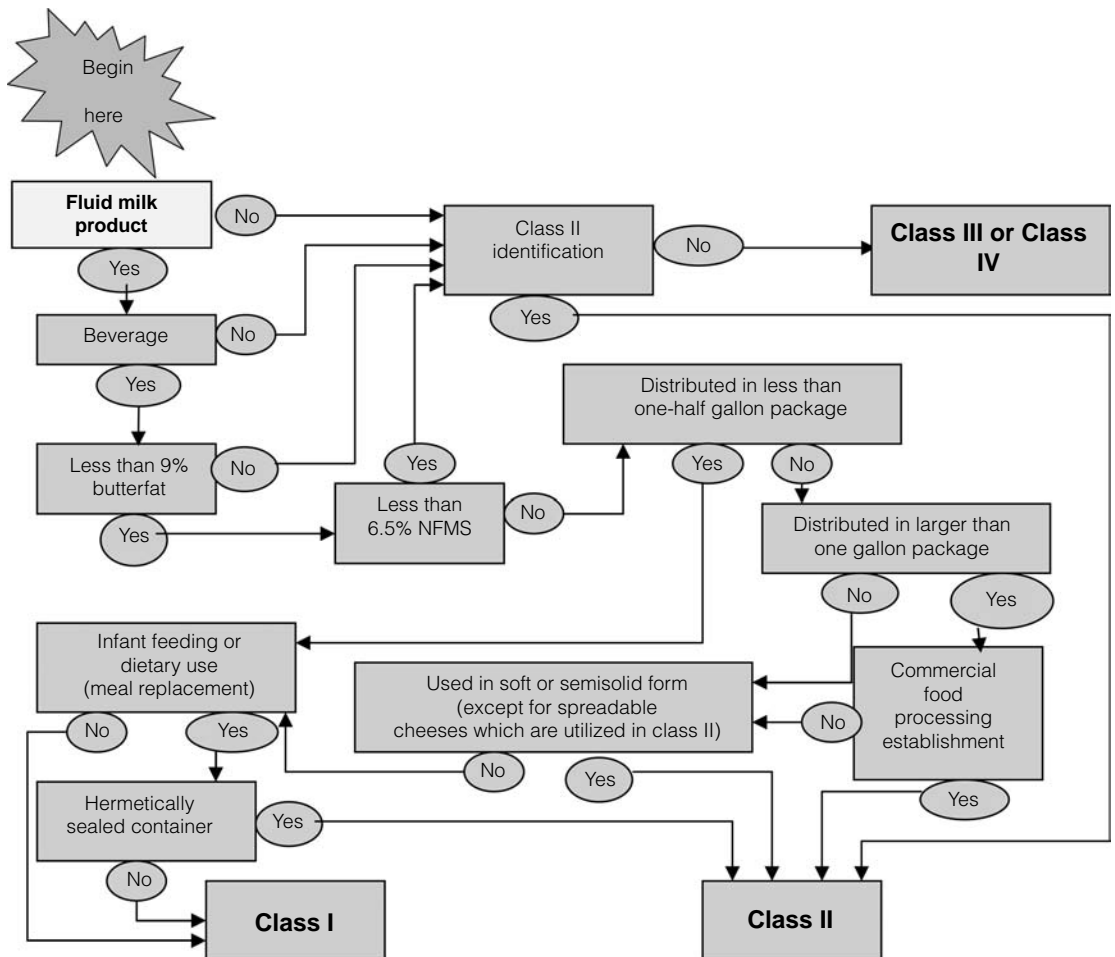


Figure 7.5. Flow diagram used to determine milk classification pricing for a product. *Source:* IDFA Milk Procurement Workbook. 2005.

than certain minimum prices for fluid milk. The price for class II, III, and IV milk is the same under all Federal Orders. Class II prices computed each month for each marketing area are based on National Agricultural Statistics Service (NASS) released prices for milk used in manufactured products. The Federal Orders also require that a plant's usage value for milk be combined with other plants usage value (pooled) and each producer (or cooperative) be paid on the basis of a uniform/blend/average price. This blend price represents an average of the value of milk in all uses (fluid milk, cottage cheese, ice cream, cheese, butter, etc.).

With federal order pooling, producers receive a common price for their milk components regard-

less of how their milk is used. Total producer milk value under the order is the sum of the following elements:

Total hundredweight milk \times Producer Price Differential (at locations)

Protein pounds \times Protein Price

Other Solids pounds \times Other Solids Price

Butterfat pounds \times Class IV/III Butterfat Price

Total hundredweight milk \times Somatic cell Adjustment

Expressed in terms of hundredweights of milk, producer prices will differ according to milk composition, milk quality, and the location of the receiving plant.

MILK PRICING FOR FERMENTED MILK PRODUCTS

Milk pricing for fermented milk and milk products is dependent if the final product will be consumed as a beverage, the level of fat and milk solids. Products similar to spoonable yogurt, sour cream are considered as class II under the Federal Milk Market Order system. Drinkable fermented products, such as cultured buttermilk, acidophilus milk, kefi, and yogurt drinks that have 6.5% or greater milk solids nonfat and less than 9% milk fat will be priced as class I. The following flow chart can be used to determine whether a product will be considered as class I or II (Fig. 7.5).

GLOSSARY

AISI 300—A quality specification for stainless steel from the American Iron and Steel Institute.

CIP (CLEANING-IN-PLACE)—A method of cleaning lines and tanks without disassembly by purging water and cleaning chemicals.

CLASSIFIED PRICING—A system used to price raw milk sold for processing based on the intended use in a specific dairy product.

COLIFORM—A group of microorganisms found in the intestinal tract; their presence indicates contamination with fecal matter.

BULK TANK UNIT—A dairy farm or a group of dairy farms from which raw milk is collected.

FDA—U.S. Food and Drug Administration.

FFD&CA (FEDERAL FOOD DRUG AND COSMETIC ACT)—An act of the U.S. Congress that specifies the basis for food safety standards.

Grade “A” PMO (PASTEURIZED MILK ORDINANCE)—Model milk regulations used for the inspection of milk production and processing facilities.

HACCP (HAZARD ANALYSIS AND CRITICAL CONTROL POINTS)—A system of steps for establishing a food safety program through identification and prevention of problems.

HHST—High temperature short time

PASTEURIZATION—A process of heating fluid milk products to render them safe for human consumption by destroying the disease-producing organisms (pathogens). The process inactivates approximately 95% of all microorganisms in milk.

IMS (INTERSTATE MILK SHIPPERS) LISTED—A publication that provides a listing

of farms and plants that have successfully passed a sanitary inspection.

NCIMS—National Conference on Interstate Milk Shipments

PHOSPHATES—An enzyme that is deactivated in milk at normal pasteurization temperatures; its presence in pasteurized milk indicates the milk has not been properly heated or was mixed with unpasteurized milk.

SINGLE-SERVICE CONTAINERS—A container used in the storage, handling, or packaging of milk or milk products intended for only one use.

SNF—Solids-notfat portion of the milk.

SOMATIC CELL COUNT—A numeric count of the dead epithelial cell and leucocytes (white blood cells) that migrate into milk from the udder of a cow.

UHT (ULTRA-HIGH TEMPERATURE)—Heat treatment at a temperature of 135–150°C for a holding time of 4–15 seconds that sterilizes the product for aseptic packaging to permit storage at ambient temperatures.

USDA—United States Department of Agriculture.

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8

Ingredients in Dairy Products

Douglas Olson and Kayanush J. Aryana

Introduction
Lactose
Nonfat dry milk
Whey products
Utilization of WPC, NFDM, and other dairy ingredients into dairy products
Conclusion
References

INTRODUCTION

Quality of dairy ingredients encompasses a variety of factors that allow it to have a long shelf life and be suitable for incorporation into high-quality dairy products. Ingredient manufacturing parameters can influence their quality. Dairy ingredients must not lead to food-borne disease hazards and must have sufficiently low bacterial and spore counts, and the concentrations of hazardous substances such as aflatoxins must meet specifications. Dairy ingredients should be stored at suitable temperatures and be kept away from pests. Also, they must meet certain requirements for chemical composition. Milk powders should flow readily and not cake. Also, various reactions such as proteolysis, lipolysis, lipid oxidation, and Maillard browning reactions must be controlled to prevent these deteriorative factors from limiting the shelf life of dairy ingredients. These ingredients should have desirable sensory properties since the sensory properties of the ingredients can affect the sensory properties of the final product incorporating these ingredients. Finally, the functional properties of the ingredients should be suitable to allow high-quality dairy products to be produced.

LACTOSE

Lactose is the most abundant milk solid ranging from 4.4 to 5.2% (Filipovitch, 1960). Lactose also called “milk sugar” (Sabin, 1884) is a disaccharide composed of α -D-glucose linked to β -D-galactose. Lactose may exist in two crystalline forms, α and β (Sharp and Doob, 1941). The α -form of lactose is lower in solubility than its β form. Solubility of lactose increases with small amounts of NaCl or KCl, exhibiting greater solubility with KCl (Gnezdilova and Kuznetsova, 1991). Undissolved lactose crystals in a product such as ice cream results in a sandy or gritty texture distinctly different from iciness because lactose crystals will not melt in the mouth (Hegenbart, 1996). Lactose crystallization influences the shelf life of frozen concentrated milk (Muir, 1984). Even at -15°C some amount of frozen milk concentrate exists unfrozen as a very concentrated solution containing lactose and milk salts. Formation of α -lactose monohydrate crystals removes free water from this solution which results in greater concentration of salts leading to milk protein instability. Prevention of lactose crystallization extends the shelf life of frozen concentrated milk.

Lactose has been known to be the dairy component that gives some dairy products a consumer disadvantage because of their lactose intolerance. Lactose intolerance is a condition where consumers possess low levels of β -galactosidase to hydrolyze lactose. Although β -galactosidase can be incorporated into fluid milk, hydrolysis of lactose can lead to changes in properties of milk, enhancing its sweetness (Choi et al., 2007). Moreover, with hydrolysis of lactose the problem of lactose crystallization no longer occurs in

concentrates, an advantage for production of frozen dairy products.

Lactose under some conditions can react with free amino groups in proteins. There is a marked loss of nutritive value since lysine, an essential amino acid, reacts with the reducing sugar lactose. The Maillard reaction (Morgan et al., 2005) is characterized by browning and it also results in flavor defects in products.

NONFAT DRY MILK

Manufacture of nonfat dry milk (NFDM) is discussed by Kelly et al. (2003). NFDM is typically prepared by evaporating skim milk in a multiple effect falling-film evaporator to 42–48% total solids before spray drying (Kelly et al., 2003). Evaporation is usually performed at 40–70°C (Birchal et al., 2005). Membrane processing such as ultrafiltration, nanofiltration, and reverse osmosis can be used as a preconcentration step in specific applications (Kelly et al., 2003). NFDM is classified as low-, medium-, and high-heat depending upon the heat treatment it receives during preheating and the amount of undenatured whey protein nitrogen that it contains. Low-, medium-, or high-heat-treated NFDM are typically preheated up to 71°C (160°F) for 2 minutes, 71–79.5°C (160–175°F) for 20 minutes, and 88°C (190°F) for 30 minutes, respectively, and contain greater than 6.0 mg/g, 1.51–5.99 mg/g, and less than 1.5 mg/g of undenatured whey protein nitrogen (American Dairy Products Institute, 2002).

Studies have been performed to determine the optimum operating parameters for spray drying to produce milk powder. A product flow rate of 1.4 kg of total solids per hour, an inlet air temperature of 160°C, and an atomization speed of 50,000 rpm in a particular spray dryer were found to be the optimum conditions for maximizing whole milk powder (WMP) quality in terms of a rapid rate of reconstitution in water (Birchal et al., 2005). Another study found that the optimum operating parameters for obtaining the best quality WMP in terms of moisture content, solubility index, and specific volume and with minimum energy consumption were using an inlet air temperature of 190°C, a feed concentration of 46% total solids, and a feed temperature of 70°C (Chauhan and Kumar, 2004). A relative humidity of $11 \pm 1\%$ and $7 \pm 1\%$ for the outlet air is needed to produce WMP and NFDM, respectively, with an a_w (water activity) of 0.20 ± 0.02 at 25°C (Schuck et al., 2005). Process control techniques for the manufacture of milk

powder should take factors such as viscosity, density, heat transfer coefficients, and stickiness under various conditions into account (O'Callaghan and Cunningham, 2005).

NFDM must contain not more than 5% moisture and not more than 1.5% milk fat (American Dairy Products Institute, 2002). It typically contains 34.0–37.0% protein, 49.5–52.0% lactose, 0.6–1.25% fat, 8.2–8.6% ash, and 3.0–4.0% moisture. The a_w for spray-dried NFDM containing 1.5, 3.0, and 4.5% moisture is 0.020, 0.100, and 0.200, respectively (Walstra and Jenness, 1984). Microbiological standards for NFDM include a standard plate count of not greater than 10,000/g, a coliform count of not greater than 10/g, and to be negative for *Salmonella*, *Listeria*, and coagulase-positive *Staphylococci* (American Dairy Products Institute, 2002). NFDM also contains 7.5–15.0 mg of scorched particles and has a titratable acidity not more than 0.15%. The solubility index of NFDM should be less than 1.2 mL except for high-heat NFDM which should be less than 2.0 mL. The color should be white to a light cream while the flavor should be clean and pleasing. NFDM should be used within 1–1.5 years.

The dispersion and reconstitution properties of NFDM can be improved by preparing instant NFDM (Kelly et al., 2003). The instantization procedure produces porous clusters of particles with diameters between 250 and 750 μm and containing entrapped air to reduce the bulk density. The moisture content of instant NFDM is typically 3.5–4.5%, and the solubility index for instant NFDM should be less than 1.0 mL (American Dairy Products Institute, 2002). Instant NFDM should be used within 6 months to 1 year. Changes in flavor can occur if temperatures reach at least 32°C (90°F) for long periods of time.

WMP is prepared from pasteurized milk which may have been homogenized. It typically contains 24.5–27.0% protein, 36.0–38.5% lactose, 26.0–28.5% fat, 5.5–6.5% ash, and 2.0–4.5% moisture (American Dairy Products Institute, 2002). WMP should be used within 6–9 months.

Viable counts of microorganisms in powdered milk samples were found to range from less than 10 to 10^3 CFU/g, and spore counts were also found at this level (Kamikado et al., 2004). Variations in microbial flora in powdered milk samples were found with different pasteurization conditions and different plants.

Thermophilic bacilli arise from spores in raw milk and form biofilm in dairy equipment before being released into the product stream (Flint et al., 2007).

Scott et al. (2007) examined the origin and type of thermophilic spores that form in WMP. Spores were predominantly formed in the preheater plate heat exchanger and the evaporator with detection beginning to occur halfway into an 18-hour manufacturing run. *Anoxybacillus flavithermus* was the predominant spore found in the preheat section, while both *Anoxybacillus flavithermus* and *Geobacillus* species were found in subsequent manufacturing stages. Although thermophiles do not pose a health threat, their presence in high numbers (greater than 10^4 CFU/g) is related to poor manufacturing practices (Flint et al., 2007). Enzymatic deterioration of the product can occur when their numbers reach 10^6 CFU/g and lead to changes in its composition and sensory properties. Therefore, milk powder should be analyzed for thermophilic bacteria. Standard methods with agar plates require 2 days. However, thermophilic bacteria in milk powders can be enumerated in 1 hour by a flow cytometry test (Flint et al., 2007). The speed of this assay would allow manufacturers to monitor their manufacturing processes.

There have been outbreaks of food poisoning traced to powdered milk. Pathogens that may have the potential to cause these outbreaks include *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Enterobacter sakazakii*.

Since *Salmonella* does not survive milk pasteurization (Marth, 1969), the presence of *Salmonella* in NFDM may be caused by improper milk pasteurization prior to drying or to post-pasteurization contamination. One outbreak of instant NFDM contaminated with *Salmonella newbrunswick* occurred in 1966 and 1967 (Collins et al., 1968). In this case, the milk was supposedly heated to 72.2°C and held at that temperature for 10–15 seconds. However, it was questionable if the milk was properly pasteurized as no thermostatic or time controls were present in the system. The milk did not receive a subsequent heat treatment sufficient to destroy the *Salmonella*. Concentration of milk was performed by a sequence of three vacuum pan evaporators, and growth of *Salmonella* may have occurred during this step. Spray drying of the concentrated milk was performed, and the powdered milk was transported with a pneumatic system to a sifting apparatus for removal of coarse aggregates. The powdered milk was then instantized (agglomerated) by subjecting the milk powder to steam and subsequently dried again to obtain larger particles that dissolve easier. Upon inspection, problems existed in the cleaning program and location of the air

intakes, and it was found that it was nearly impossible to properly clean some parts of the spray dryer and instantizing system. As a result, inaccessible areas in the after dryer had buildup of caked material. Two workers in the sifting and bagging operation were determined to be infected with *S. newbrunswick*. The instantizing process was found to be the source of *Salmonella* in the plant.

Hobbs (1955) reported outbreaks of typical staphylococcal food poisoning that occurred in 1953. *S. aureus* can be present in raw milk, and its growth can be promoted by long prestorage at ambient temperature and by inefficient preheating. Milk can become contaminated with *S. aureus* by plant personnel. *S. aureus* and its enterotoxin can be introduced into evaporated milk by sludge formed on the bottom of an atomizer feed tank, and this contamination would likely become more severe during a temporary stoppage of spray drying. No competition from other microorganisms would likely lead to further growth of *S. aureus*. Although the number of *S. aureus* is greatly reduced during spray drying, small numbers of *S. aureus* can survive and enterotoxin can remain, leading to food safety hazards.

L. monocytogenes in both skim milk and concentrated skim milk can survive spray drying but with a 1–1.5 log reduction in viable counts (Doyle et al., 1985). However, a greater reduction (more than 4 log CFU/g) in cell counts occurred during 16 weeks of storage at room temperature in their study.

Much research has been performed with *Bacillus* in milk powder. Viable cells and spores of *B. cereus* are frequently found in milk powder in low numbers ($<10^3$ CFU/g) (Reyes et al., 2007). Floristean et al. (2004) isolated *B. cereus* from 37.5% of the powdered milk samples that they analyzed. Costa et al. (2004) surveyed the occurrence of *B. cereus* in powdered milk samples in Brazil and found that 13 out of 75 powdered milk samples contained *B. cereus* and 3 of these exceeded the maximum limit allowed by their legislation. Pavic et al. (2005) reported a food poisoning outbreak involving toxigenic *B. subtilis* and *B. licheniformis* present in milk powder. They showed that both of these species could begin the log phase of growth in reconstituted milk within 2 hours of storage at room temperature.

E. sakazakii, considered to be an opportunistic pathogen, has been found in powdered milk in one study (Iversen and Forsythe, 2004), but not in a more recent study (Shaker et al., 2007). There is much concern about the presence of this organism in powdered infant formula milk (Iversen and Forsythe, 2004).

PCR (polymerase chain reaction) methods have been developed to detect pathogens in NFDM. *Salmonella* in milk powder can be detected on the basis of real-time PCR methods (Malorny et al., 2007). Although no viable *S. aureus* was detected in NFDM from the large-scale food poisoning outbreak in Japan, PCR was used to detect *sea* and *nuc* genes in *S. aureus* (Ikeda et al., 2005). Cooper and McKillip (2006) described a procedure for detecting enterotoxigenic *Bacillus* species in NFDM using rep-PCR. PCR has also been used to detect *E. sakazakii* in powdered milk (Xu et al., 2006).

The presence of aflatoxin in milk powder is a concern. Deveci and Sezgin (2005) found that aflatoxin M₁ levels in NFDM produced in Turkey ranged from 0 to 0.705 µg/kg and that the aflatoxin M₁ contents were higher in the winter than in the summer. Mean levels of 0.056 µg/L of aflatoxin M₁ were found in goat milk powder from Brazil (Oliveira and Ferraz, 2007). However, no samples of milk powder were found to be contaminated with aflatoxin M₁ in the study of Martins et al. (2005). Jasutienė et al. (2007) artificially contaminated dissolved milk powder with 0.044 mg/g of aflatoxin M₁ and found that pasteurization at 95°C for 3 minutes and milk fermentation did not significantly affect aflatoxin M₁ stability.

Pests are often a problem with NFDM. The subject of insect infestation in various animal products including milk powder and their control has been reviewed by Rajendran and Parveen (2005). These authors listed pests including several types of beetles that can breed in the presence of milk powder, and they discussed the use of phosphine for insect control in milk powder. Abd El-Halim et al. (2006) reported that milk powder was the most suitable substrate for the *Caloglyphus redikorzevi* mite in Egypt. In addition, flies and cockroaches can also infect dairy plants (Hall and Hedrick, 1966).

Recent research has been performed on rapid methods of analysis and methods for measuring free fat in milk powder. Powdered milk can be rapidly analyzed for nutritional components by their distinct fingerprint characteristics obtained by Fourier transform infrared spectroscopy (Deng et al., 2007). Lipids can be analyzed by the C=O bond at 1,747/cm and the CH₂ bonds at 2,854/cm and 2,925/cm, and proteins can be analyzed by C=O bonds at 1,658/cm and by N-H and C-N bonds at 1,540/cm. Carbohydrates can be analyzed by C-O bonds at 1,200–900/cm. The protein content in several milk powder samples can be precisely determined simultaneously by a digital image determining method (Hou and Sun,

2005). Shibata et al. (2006) reported the optimal conditions for measuring free fat in milk powder, and they found that *n*-hexane can be used as a suitable solvent.

Clarke and Augustin (2005) found that the solvent-extractable fat content in milk powder can be increased by separating milk into cream and skim milk, pasteurizing the cream, and possibly homogenizing the cream at a high temperature and pressure before combining it with skim milk concentrate. The concentrate is spray dried without homogenization. Also, the total solids content of the cream and the milk concentrates used to produce the milk powders also influenced the solvent-extractable fat level in milk powder. It is desirable to have a high solvent-extractable fat content in milk powder that will be used for chocolate manufacture.

Both lactose and fat are involved in the flowability and caking of milk powder. Amorphous lactose in spray-dried milk powders absorbs moisture and leads to reduced flowability and to caking (Fitzpatrick et al., 2005). The stickiness and caking sensitivity of dairy powders during drying and storage can be predicted on the basis of subtracting the glass transition temperature (T_g) from the product temperature and using the heat capacity changes during the glass transition (Schuck et al., 2005). Flowability of spray-dried milk powders decreases with increasing fat content but increases with increasing particle size (Fitzpatrick et al., 2005). The caking of dairy powders during storage occurs when liquid bridges of fat partially solidify on the surface due to cooling of the powders (Foster et al., 2005). The cohesiveness of the powders does not increase in the presence of only liquid fat bridges. Therefore, fluctuating storage temperatures of packaged powders should be avoided when surface fat contents are greater than 13% (w/w) (total fat content of 41% [w/w]) (Foster et al., 2005). Also, powder-handling equipment such as hoppers and silos must be properly designed to allow proper milk powder flowability (Fitzpatrick et al., 2005).

The processing of raw milk into WMP lowers its heat stability (Negri et al., 2004). They also reported that heat coagulation times of WMP (and the raw milk used to produce this powder) were higher during the summer than during the spring.

Residual enzyme activity has been detected in milk powder. Thermostable lipases from microbial sources lead to deterioration of WMP during storage and limit the shelf life, and the rancid flavor of WMP mainly arises from the presence of short-chain

free fatty acids (Páez et al., 2006). Alkanhal (2006) found that the percent of remaining milk lipase activity in milk powder was 1.7, 0.5, and 0.2% and the percent of remaining milk protease activity was 12.3, 2.8, and 0.4% for low-, medium-, and high-heat powders, respectively, while 18.2, 6.25, and 3.5% of the microbial proteolytic activity remained in low-, medium-, and high-heat powders, respectively. However, unlike the study of Páez et al. (2006), Alkanhal (2006) reported that microbial lipases were completely inactivated. Quality deterioration of recombined ultrahigh temperature milk products can occur during storage from the residual activity of these lipases and proteases in milk powder (Alkanhal, 2006). Changes in free fatty acids in WMP were related to the season of manufacture of the WMP rather than to the storage temperature and time (Páez et al., 2006).

Many deteriorative reactions can occur during storage of milk powder including lactose crystallization, lipid oxidation, and Maillard browning reactions. The occurrence of these deteriorative reactions is interrelated (Thomsen et al., 2005). The preheat treatment of milk, a_w , and storage temperature affect the shelf life of WMP (Stapelfeldt et al., 1997). Temperatures above T_g allow the crystallization of amorphous lactose (Jouppila and Roos, 1994). Oxidation may be promoted by an increase in the free fat content that could result from lactose crystallization in milk powder (Stapelfeldt et al., 1997). Low-heat milk powder is more susceptible to severe oxidative changes and nonenzymatic browning. The autoxidation rate increases with a_w in the range of 0.11–0.33. However, oxidation can occur in milk powder with an extremely low moisture content due to the ease at which these particles can disintegrate leading to increased exposure to air (Stapelfeldt et al., 1997). WMP will undergo oxidative changes at a storage temperature of 40°C as measured by thiobarbituric acid-reactive substances and free fat concentration, but oxidized flavor development is delayed by the Maillard reaction (Páez et al., 2004/2005). Nonenzymatic browning is also promoted at higher a_w and higher storage temperatures leading to less desirable sensory properties (Stapelfeldt et al., 1997).

Ideally, fresh NFDM should have a mild and bland flavor characteristic of skim milk. Nonanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, γ -undecanolactone, γ -dodecalactone, δ -decanolactone, and δ -undecalactone have a sweet and milky smell and are found in spray-dried NFDM, while tetradecanal and β -ionone are the offensive

smell compounds in spray-dried NFDM (Shiratsuchi, 2004). Concentrations of some volatile compounds such as *n*-pentanal, *n*-hexanal, dimethyl sulfide and butyric acid detected in WMP varies with season, while concentrations of other volatile compounds, such as *n*-heptanal, *n*-octanal, *n*-nonanal, and 3-methyl butanal, in WMP, do not vary with season (Biolatto et al., 2007). Milk powder tends to develop an oxidized/stale flavor during long-term storage, especially at high storage temperatures (Driscoll et al., 1985). Off-flavors present in NFDM used as an ingredient can be transferred into the final product, and the extent to which these off-flavors can be masked in the final product varies (Caudle et al., 2005).

Milk powders can be fortified with calcium (Williams et al., 2005). However, the color, texture, stability, flavor, and processing characteristics of the final product may be potentially influenced by the form of calcium used for fortification

WHEY PRODUCTS

The old view of whey as a cheesemaking by-product that needs to be disposed causing environmental concerns has been replaced by a new view that whey should be utilized to produce valuable, high-quality ingredients. Peters (2005) evaluated the economic feasibility of producing whey products in different cheese plant sizes, and the size of the plant has a large effect on the returns. In 2000, 1.8 million tonnes of whey powder and 400,000 tonnes of demineralized whey powder were produced in the European Union and the United States (Foegeding and Luck, 2003). Improvements have been made in the quality of whey protein products, especially if it is obtained prior to cheesemaking instead of obtained after cheesemaking. Nelson and Barbano (2005) produced a serum protein concentrate that did not contain rennet, culture, color, and lactic acid from cheesemaking by concentrating permeate from microfiltered skim milk by ultrafiltration. A relative humidity of the outlet air of $7 \pm 1\%$ is needed to produce whey powder with an a_w of 0.20 ± 0.02 at 25°C. The production of various types of whey products is summarized by Mulvihill and Ennis (2003).

Whey products include dry sweet-type whey, dry acid-type whey, reduced lactose whey, reduced minerals whey, whey protein concentrate (WPC), whey protein isolate (WPI), and various modified and fractionated products. Dry sweet-type whey is produced by drying the whey from rennet-coagulated cheeses such as Cheddar cheese while acid-type whey is

produced by drying the whey from acid-coagulated cheeses such as Cottage cheese and Ricotta. The ash content (9.8–12.3%) and titratable acidity (0.35–0.44%) of acid-type whey are higher than the ash content (8.2–8.8%) and titratable acidity (0.10–0.15%) of dry sweet-type whey (American Dairy Products Institute, 2002). Also, the lactose content of dry acid-type whey (61.0–70.0%) is likely to be lower than the lactose content of dry sweet-type whey (63.0–75.0%), especially, if starter bacteria are used to produce acid in the acid-coagulated cheese. Similar to NFDM and WMP, whey products contain a scorched particle content of 7.5–15.0 mg. Reduced lactose whey is produced by selectively removing lactose by precipitation, filtration and dialysis so that the final lactose content does not exceed 60%, and reduced mineral whey is produced by removing minerals by precipitation, filtration and dialysis so that the final ash content does not exceed 7%. WPC is produced by ultrafiltration and diafiltration to concentrate the whey prior to spray drying so that the final product contains 34.0–80.0% protein, 10.0–55.0% lactose, 1.0–10.0% fat, 4.0–8.0% ash, and 3.0–4.0% moisture. WPI is produced by various ion exchange techniques prior to spray drying to obtain a final product that typically contains 92.0% protein, 0.5% lactose, 1.0% fat, 2.0% ash, and 4.5% moisture. Microbiological standards for whey products include a standard plate count of not greater than 30,000/g, a coliform count of not greater than 10/g, and to be negative for *Salmonella*, *Listeria*, and coagulase-positive *Staphylococci*. Although there is some variation in shelf life depending on the particular type of whey product, they should be used within 6 months to 1 year.

Whey proteins and their products have desirable functional properties including water binding, emulsification, foam formation, and gel formation upon heating. However, much variation in composition and functional properties of commercial WPC occurs because of variations in cheese–milk composition, whey composition from different types of cheeses from different plants and different lots within the same plant, whey storage time and temperature, and processing conditions (Morr, 1992). Their functional properties have been reviewed by Morr and Ha (1993), Singh (2003), Mulvihill and Ennis (2003), Dickinson (2003), Singh and Havea (2003), and Carr et al. (2003). Moon et al. (2007) showed that viscosity of 10% WPC suspensions and gel formation of WPC differed on the basis of the drying method.

UTILIZATION OF WPC, NFDM, AND OTHER DAIRY INGREDIENTS INTO DAIRY PRODUCTS

Much research has been performed on investigating the quality of dairy products supplemented with dairy ingredients. Although dairy ingredients have been incorporated into many food products, incorporation only into dairy products will be discussed in this section. Dairy ingredients accounted for utilization of 50% of the WPC produced in 1999 (Foegeding and Luck, 2003). WPC has been used in many different types of dairy products including various types of cheese, yogurt, ice cream, frozen yogurt, dairy spreads such as butter and low-fat butter, infant formula, cream liqueurs, creamy chocolate dairy desserts, *peda* and *gulabjamun* (Indian dairy products derived from *khoa*), and *kulf* (a traditional frozen dairy product). WPC is commonly used as a fat replacer in many types of dairy products. Milk powder has been investigated in many of these products as well as in *shrikhandwadi* and *Labneh*.

Work has been performed on WPC incorporation into many types of cheese. Baldwin et al. (1986) manufactured Cheddar cheese with WPC (heated to 70°C for 15 minutes) added to whole milk. The curd structure was weakened by addition of WPC. Cheese made with added WPC had a lower total solids content than the control probably due to a high water binding capacity of WPC. This cheese had more flavor defects with atypical or unclear cheese flavor being the most common criticism, and some of the cheese made with added WPC was slightly sticky or pasty. When comparing low-fat Mozzarella cheeses (6% fat) prepared by preacidification of milk, by combining preacidification with the use of exopolysaccharide producing starters, and by combining preacidification with WPC, Zisu and Shah (2005) found that incorporating WPC led to the softest cheeses and to the least increase in meltability over time. Also, surface scorching was not reduced and fusion of cheese shreds was not increased when incorporating WPC into low-fat Mozzarella. However, these properties improved upon aging the cheeses between day 7 and 45 (Zisu and Shah, 2005). Lobato-Calleros et al. (2007) made white fresh cheese-like products by replacing milk fat with WPC and found that these cheese-like products containing WPC had a close and compact network consisting of short-linking milk protein strands. Ibrahim et al. (2001) manufactured a low-fat soft cheese containing WPC and found that this cheese

had an acceptable firmness a smooth texture, and the consistency of cheese spread. Veiga et al. (2000) collected six commercial brands of Brazilian Petit Suisse cheeses from local markets and found that the product that had a water-holding capacity more than twice as much as the remaining samples contained WPC. WPC has been used as a fat substitute in hard Requeijão cheese with a reduced fat content (Soares et al., 2002). Gigante et al. (2001) found that adding WPC to Requeijão cheese increased its firmness and decreased its meltability. The latter effect was greatest when the milk was preacidified to pH 5.2 prior to ultrafiltration to form the cheese base for the Requeijão cheese. El-Sheikh et al. (2001) reported that low-fat Domiati cheese (2.1% fat) containing particulated WPC (5%) received flavor scores that were close to full-fat Domiati cheese and higher body/texture scores indicating that particulated WPC is an effective fat replacer in low-fat Domiati cheese. Lobato-Calleros et al. (2001) reported that low-fat Mexican Manchego cheese incorporating WPC as a fat replacer had similar sensory properties and similar hardness, springiness, cohesiveness, and chewiness compared to full-fat Mexican Manchego cheese. Lobato-Calleros et al. (2001) also found that the microstructure of low-fat Manchego cheese incorporating WPC did not have the compact and dense protein matrix that was present in the control low-fat Mexican Manchego cheese.

WPC has been used in processed cheese, processed cheese analogues, and processed cheese spreads. Kaminarides and Stachtariis (2000) manufactured processed cheese mainly from kasseri cheese without either WPC or soybean oil and manufactured processed cheese analogues with increasing amounts of WPC and soybean oil incorporation. They found that the processed cheese analogues were softer and more spreadable than the processed cheese control. More deterioration of flavor during storage occurred in the processed cheese analogues containing the highest amount of WPC and soybean oil than in the other products. Abd-El-Salam et al. (1998) found that the increasing concentration of WPC in processed cheese spread led to increased whiteness in the spread.

Variable results have been found for effect of WPC incorporation on growth of cultures in yogurt. Christopher et al. (2006) reported that viability of *Bifidobacterium bifidum* strains in yogurt was significantly improved with WPC incorporation at 0.5 and 1.0% in yogurt. Reddy et al. (2005) found that the incorporation of WPC into set and stirred yogurt led to increased viability of *Lactobacillus aci-*

dophilus compared to the control yogurt. However, Cheng et al. (2006) found that *Lactobacillus* counts in stirred yogurts were not affected by incorporating 90:10 and 80:20 combinations of WMP to WPC into yogurt compared to yogurts containing WMP instead of WPC.

The pH and titratable acidity of yogurt are affected by WPC. Cheng et al. (2006) reported lower pH and acidity variations in stirred yogurts containing 90:10 and 80:20 combinations of WMP to WPC than in yogurts not containing WPC. Incorporating WPC into yogurt led to smaller decreases in pH during storage (Christopher et al., 2006; Reddy et al., 2005). Titratable acidities of yogurts tended to increase with the presence and level of WPC probably due to buffering capacity of proteins (Modler et al., 1983).

Rheological properties of yogurt are often improved upon WPC incorporation. WPC incorporation led to increased hardness and gumminess in yogurt (Antunes et al., 2004) and to increased firmness, hardness, and adhesiveness in yogurt made from goat's milk (Herrero and Requena, 2006), and these properties of goat milk yogurt were maintained during its shelf life. Gel firmness in yogurt increased when WPC concentration increased from 0.5 to 1.5% (Modler et al., 1983). Raziuddin et al. (2004) found that 1.0 and 1.5% WPC incorporation in low-fat yogurt improved the curd tension and viscosity, and Cheng et al. (2006) found that stirred yogurts containing 90:10 and 80:20 combinations of WMP to WPC had a higher viscosity and a better structure than the yogurt containing only WMP. A higher water-holding capacity (Cheng et al., 2006; Sodini et al., 2005) and decreased syneresis (Antunes et al., 2004; Raziuddin et al., 2004) were found when WPC was incorporated into yogurt. Syneresis in yogurt tended to decrease when increasing WPC level (Modler et al., 1983).

Other studies have shown that WPC incorporation leads to a yogurt with less desirable rheological properties. Yogurts containing WPC were usually less firm than yogurt containing sodium caseinate (Modler et al., 1983). Also, yogurts containing 1.5% WPC had more syneresis than yogurts containing 1.5% sodium caseinate, and the 0.5% gelatin control in their study had much less syneresis than yogurts containing WPC. Modler and Kalab (1983) explained the tendency for softer gels and more syneresis in yogurts containing 1.5% WPC compared to yogurts containing 1.5% of other proteins by the much lower extent of micellar fusion.

Variable results have been found for the effect of WPC incorporation on sensory properties of yogurt.

Many studies have shown that sensory properties and overall acceptability of yogurts containing moderate levels of WPC are comparable to their control (Antunes et al., 2004; Christopher et al., 2006; Raziuddin et al., 2004; Reddy et al., 2005). However, the sensory properties of yogurt have been reported to be adversely affected by levels higher than 1.5% WPC incorporation (Raziuddin et al., 2004) and higher than 0.5% WPC incorporation (Christopher et al., 2006). Unlike other studies, Cheng et al. (2006) found that the replacement of 10–20% of the WMP with WPC improved the fl vor. Increasing WPC concentration in yogurt resulted in higher sensory firmnes scores (Modler et al., 1983).

Lee et al. (1990) compared milk yogurt to soymilk yogurt, both containing WPC. The milk yogurt was whiter and less viscous than the soymilk yogurt. Flavor scores were higher for milk yogurt than for soymilk yogurt due to greater acidity intensity in milk yogurt and the beany and other off-fl vors present in soymilk yogurt. Also, milk yogurt had slightly higher body/texture scores.

Many whey products including sweet whey, reduced-lactose whey, demineralized whey, modified whey, WPC, and WPI are used in ice cream (Chabelski and Castellanos, 2004). When including whey products in ice cream, lactose crystallization must be prevented and salty fl vor arising from whey incorporation needs to be taken into account. Lightness of the ice cream mix decreased when the whey solids to skim milk ratio increased, but this color difference was not visually detected after freezing (Huse et al., 1984). Tirumalesha and Jayaprakasha (1998) replaced skim milk solids with a blended 50:50 mixture of spray-dried WPC and sweet cream buttermilk powder at 25, 50, 75, and 100% levels and found that an increasing level of substitution led to a slight increase in titratable acidity, a slight decrease in pH, decreased freezing point, and decreased viscosity of the mix and increased overrun, decreased hardness, and decreased melting resistance of the ice cream. Ruger et al. (2002) reported that substituting 1% NFDM with 1% WPC in ice cream mix did not affect mix viscosity as measured by fl w time through a pipette. Viscosity of ice cream mix decreased as the WPC level increased (Huse et al., 1984). Khillari et al. (2007) found that the overrun and melting quality were not reduced upon replacement of 20% of the fat with WPC, but there was a progressive decrease in ice cream mix viscosity with increased replacement of fat with WPC. Udabage et al. (2005) manufactured ice cream containing low-heat NFDM, WPC

with 35% protein (WPC 35), and unheated and heated blends of low-heat NFDM and WPC 35 and found that the ice cream with the best melt resistance was prepared by using the high-heat-treated blend of low-heat NFDM and WPC 35. However, Lee and White (1991) found that meltdown resistance did not depend on the presence or level of WPC in ice cream. At high concentrations of WPC, penetration depth decreased meaning that firmnes increased (Huse et al., 1984).

Many, but not all, studies have shown that WPC can be incorporated into ice cream without adversely affecting the sensory properties. Khillari et al. (2007) reported that incorporation of WPC to replace up to 40% of the fat did not adversely affect the sensory quality of ice cream. Patel et al. (2006) reported that an acceptable high-protein ice cream containing either smaller or similar-sized ice crystals can be produced by incorporation of WPC or milk protein concentrate powders. Ruger et al. (2002) reported that substituting 1% NFDM with 1% WPC in ice cream mix did not improve the texture of the ice cream as measured by mean ice crystal size and sensory evaluation. Parsons et al. (1985) manufactured ice creams either using NFDM as a control or using WPC, a blend of WPC and dry sweet whey, and a blend of dry sweet whey and sodium caseinate to replace either 50 or 100% of the milk solids-not-fat. They found that there were no significant differences in fl vor and body/texture among the ice creams when evaluated by an expert panel and no significant differences in fl vor among the ice creams except for a significantl poorer fl vor for the ice creams made with dry sweet whey and sodium caseinate blend when evaluated by consumers. Tirumalesha and Jayaprakasha (1998) replaced skim milk solids with a blended 50:50 mixture of spray-dried WPC and sweet cream buttermilk powder at 25, 50, 75, and 100% levels and found that an increasing level of substitution had no significant effect on sensory properties. An ice cream mix containing 10% solids-not-fat, a 50:50 blend of skim milk to whey solids, and 72% hydrolyzed lactose syrup obtained the highest score in creaminess, smoothness, and fullness of fl vor (Huse et al., 1984). The average sensory scores for smoothness, creaminess, and fullness of fl vor of ice cream made from solids-not-fat consisting of 100% skim milk and made from solids-not-fat consisting of 50% skim milk and 50% whey solids were similar, but these scores decreased for ice cream made from solids-not-fat containing 100% whey solids. In these latter mixes, a

very fl and increased cooked fl vor was noticed because of the high concentration of the heat-sensitive whey proteins (Huse et al., 1984). Lower fl vor and body/texture scores were obtained for ice creams containing WPC compared to their control (Lee and White, 1991). The high lactose concentration of ice cream containing WPC probably caused these samples to be judged as coarse, icy, and weak. However, fl vor and body/texture scores for heat-shocked ice creams with or without WPC as an ingredient were similar (Lee and White, 1991).

Replacing NFDM with acetylated WPC in chocolate ice milk mix led to an increase in moisture, total protein, nonprotein nitrogen, and ash contents, viscosity, and density of the mixes and increase in melting resistance in the frozen product. When replacing up to 50% of the NFDM with acetylated WPC, both the overrun and the sensory scores of the chocolate ice milk increased, but a 75 and 100% replacement decreased both the overrun and the sensory scores. Therefore, Khader et al. (2001) concluded that up to a 50% substitution of NFDM with acetylated WPC did not negatively impact chocolate ice milk quality.

WPC has been incorporated into frozen yogurt mixes. Hamed et al. (2004) manufactured frozen yogurt with NFDM incorporation and with WPC replacing 50, 75, and 100% of the NFDM in the mix, while Jayaprakasha et al. (2000) used WPC to replace 10–90% of the NFDM in the manufacture of frozen yogurt. Hamed et al. (2004) found that replacing NFDM with WPC increased the melting resistance of the frozen yogurt and enhanced the growth of *bifidobacteria*. Both Hamed et al. (2004) and Jayaprakasha et al. (2000) reported that the optimum level of NFDM replacement with WPC in frozen yogurt was 50%. Hamed et al. (2004) reported that this frozen yogurt received the highest fl vor scores. Jayaprakasha et al. (2000) found that this frozen yogurt developed a desirable acidity quicker than the control, had less whey separation and a better body/texture and mouth-feel than the control, and attained a higher maximum overrun compared to the control. Opdahl and Baer (1991) manufactured frozen yogurt using WPC and a fermented WPC with a mix composition of 6% milk fat, 10.5% WPC, 11% sucrose, 3% corn syrup solids, and 0.3% stabilizer and emulsifier blend with a final mix titratable acidity of 0.43% and a pH range of 4.70–5.30. A survey revealed 87.8% of the people who sampled this product liked it and 81.2% claimed that they would purchase this product if the price was the same as ice cream. An acceptable frozen yogurt

can be made when WPC and fermented WPC replace all of the milk solids-not-fat.

Jensen et al. (1987) reported that sodium caseinate, WPC, and ultrafiltration retentate from skim milk can be incorporated into dairy spreads. These proteins help form a stable emulsion, bind water, and help create a desirable texture and spreadability.

Fatma et al. (2005) prepared an infant formula with a commercial WPC and with WPC prepared by 4× ultrafiltration 5× ultrafiltration 4× diafiltration and 8× diafiltration using a WPC to sodium caseinate ratio of 40:60. The infant formulas were analyzed for emulsion volume index, viscosity, protein solubility, sedimentation, and particle size. It was concluded that the most desirable WPC preparation for manufacturing infant formula was WPC prepared by 8× diafiltration.

Kaustinen and Bradley (1987) prepared cream-based liqueurs by using demineralized WPC as an ingredient. Since WPC is an effective emulsifier in certain applications, it was used to prevent fat separation after homogenization. When making cream-based liqueurs with washed cream as the fat source and by adding ethanol before homogenization, the product was stable to visible separation of fat at 40°C for 90 days. Cream-based liqueurs prepared by this method had similar coffee-fl vor intensity, sweetness, texture, smoothness, overall preference, less off-fl vors and alcohol-fl vor intensity, and a thinner body compared to a commercial cream liqueur.

Nikaedo et al. (2004) investigated the optimum formula for a creamy chocolate dairy dessert. Levels of ingredients used to manufacture these desserts included 1.0, 2.0, and 3.0% WPC; 5.0, 6.0, and 7.0% WMP; 0.05, 0.15, and 0.25% carrageenan; 0.05, 0.15, and 0.25% guar gum; 2.8% cocoa powder; 11.5% sugar; and 0.08% potassium sorbate. The formulation that was considered the most acceptable among the panelists was the dessert that included 1% WPC, 7% WMP, 0.05% carrageenan, and 0.25% guar gum.

Dewani and Jayaprakasha (2002) manufactured *peda* and *gulabjamun* from milk and ultrafiltration whey retentate in proportions of 20:80, 30:70, 40:60, and 50:50. They found increasing color and appearance, body/texture, and overall acceptability scores with increasing WPC level up to 40% and increasing fl vor scores with increasing WPC level up to 50% in *peda*. *Gulabjamun* containing 30% WPC was found to be comparable to their control.

Jayaprakasha et al. (1999) prepared *kulf* by using WPC to replace 20–100% of the skim milk solids. Increasing the incorporated WPC level led to a

significant increase in the freezing point depression and mix viscosity, improved mouthfeel, and better melting resistance. These authors concluded that up to 80% of the skim milk solids can be replaced with WPC without adversely affecting the quality of *kulfi*.

Research has also been performed on the effect of incorporation or replacement of milk powder or powdered skim milk retentate on the quality of the resulting product. Bramhapurkar et al. (2007) reported that fortifying *shrikhandwadi* with 10% NFDM enhanced its quality, but fortification with 15% NFDM tended to adversely affect its sensory quality. Fortification of Labneh with NFDM resulted in Labneh with a strong elastic structure (Kossah et al., 2004). Hardness and stickiness of Labneh decreased considerably with an increase in milk solids concentration (Kossah et al., 2004). Danków and Cais-Sokolińska (2002) manufactured ice cream in which the NFDM was replaced with high-protein milk concentrate and found that the resulting ice cream had increased aeration, viscosity, resistance to melting, and nutritional value. The amount of stabilizer and emulsifier incorporated into the ice cream could be decreased with the use of high-protein milk concentrate. Domiati cheese manufactured from milk fortified with 10% NFDM had higher moisture, protein, and calcium contents than Domiati cheese made from control milk (Ibrahim et al., 1999). A probiotic fat-free soft Kareish cheese was manufactured from powdered skim milk retentate from ultrafiltration and found to have high-acceptability scores after storage for 15 days (Younis, 1998).

CONCLUSION

Dairy ingredients including various whey products (sweet whey, reduced-lactose whey, demineralized whey, WPC, fermented WPC, acetylated WPC, particulated WPC, and WPI), various milk powders (NFDM, WMP, and powdered skim milk retentate), milk protein concentrates, sodium caseinate, and sweet cream buttermilk powder have been used or studied for their suitability for incorporation into various dairy products including various types of cheese, yogurt, ice cream, frozen yogurt, dairy spreads such as butter and low-fat butter, infant formula, cream liqueurs, creamy chocolate dairy desserts, *peda* and *gulabjamun*, and *kulfi*. Variable results for various properties of these dairy products during its shelf life have been obtained. Therefore, manufacturers must be selective in using the proper types of high-quality ingredients for their particular application to obtain high-quality manufactured dairy products.

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9

Fluid Milk Products

John Partridge

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INTRODUCTION

Fluid milk is the natural first food for infants of any mammalian species. Historically, man has been able to domesticate several species of mammals for the express purpose of producing and harvesting milk for human consumption. Cows and goats are the predominant producers of milk for fluid consumption, with cow's milk being by far the larger component of the fluid milk industry worldwide. For effective discussion of the fluid milk process, unless otherwise noted, cow's milk will be the object of this chapter.

Milk for beverage use is available almost anywhere one might travel with variations in delivery methods ranging from a dipper in tinned can with 1–2 day of shelf life to aseptically handled and packaged products with 6 months or more of shelf life. According

to preliminary data for 2007 collected by the USDA, consumption of milk for fluid beverage use varies from as low as 2.3% of the total milk production in New Zealand to as high as 104.6% of total production in India requiring imported product to fill the need. The United States will use approximately one third of 85+ million metric tons of milk produced in 2007 for fluid consumption (USDA, 2007a). Unlike more shelf-stable milk products such as cheese, powders, and even ice cream, fluid milk is primarily sold within a state or national geographical area rather than a larger regional or global market.

In the United States, the regulation of the fluid milk industry is built around the Grade "A" Pasteurized Milk Ordinance (PMO; USHHS-FDA, 2003a). The United States Public Health Service (USPHS) began assisting states and municipalities in the prevention of milk-borne disease by developing the first model regulation in 1924. The National Conference on Interstate Milk Shipments (NCIMS) is held every 2 years and offers an opportunity for regulatory, industry, and academic representatives to meet and continuously refine the PMO based on the latest data and research. All 50 states, the District of Columbia and U.S. Territories use the PMO as the model for regulation. Oversight by USPHS/Food and Drug Administration (FDA) provides for uniform standards and interpretation thus removing barriers to interstate commerce. Standards differ within countries so this chapter will utilize the standards set in the USPHS-PMO and the U.S. Code of Federal Regulations (CFR) when addressing regulatory issues.

Milk is legally defined as:

Milk is the lacteal secretion, practically free from colostrum, obtained by the complete milking of one

or more healthy cows. Milk that is in the final package form for beverage use shall have been pasteurized or ultra-pasteurized, and shall contain not less than 8 $\frac{1}{4}$ percent milk solids not fat and not less than 3 $\frac{1}{4}$ percent milkfat. Milk may have been adjusted by separating part of the milkfat therefrom, or by adding thereto cream, concentrated milk, dry whole milk, skim milk, concentrated skim milk, or non-fat dry milk. Milk may be homogenized. (USHHS-FDA, 2007a)

Colostrum is the first milk removed from the mammary gland after parturition of the calf and is abnormally high in blood components, especially immunoglobulins that are important for transferring passive immunity to the newborn calf. Producers must withhold this milk from the commercial supply. Milk from animals that show signs of mastitis or other health issues should also be withheld from the milk supply. This includes milk from an animal that has been treated with an antibiotic. The quantity and quality of milk will be maximized in animals that are in excellent health, therefore, appropriate herd health programs must be an important part of the producers' operations. For further information about regulations for product standards and labeling as well as for quality and safety standards, see Chapters 6 and 7.

FLUID MILK PRODUCTS

Beyond the whole milk minimums for milk fat and milk solids-not-fat (MSNF) there are a multitude of adjustments that can be made to fluid milk. The Code of Federal Regulations further stipulates that milk designated for consumption by school lunch programs will contain the levels of vitamins A (2,000 IU/quart) and D (400 IU/quart) that are specified by FDA in Title 21 CFR Part 210.10(m)(1)(ii) (USDA, 2007b). The requirement for vitamin D addition to milk for school lunch programs effectively results in the addition of vitamin D to most of the fluid milk on the market. By adding vitamin D to all products bottled within a facility, the plant avoids the logistical nightmare of keeping supplemented and unsupplemented products separated during the processing, packaging, and distribution. The addition of vitamin A is mandatory whenever a standardization process reduces the fat content of the milk below the 3 $\frac{1}{4}$ % (whole milk) level. Since school lunch programs primarily serve skim, low fat, and reduced fat milk products the school children will receive both vitamin A and D supplements in their milk. If vitamins are added to the milk, the term "vit. A," "vitamins A and D" or

a similar declaration must be included on the label. Modification to the fat level of milk may be labeled according to the guidelines of the National Labeling and Education Act (NLEA). The regulations for nutrient content claims for fat may be found in Title 21 CFR Part 101.62 (USHHS-FDA, 2007b). Reducing fat by at least 25% from the whole milk reference allows the label to use the terms "reduced fat," "reduced in fat," "fat reduced," "less fat," "lower fat," or "lower in fat." The most common product that falls in this category is 2% milk. The terms "low fat," "low in fat," "contains a small amount of fat," "low source of fat," or "little fat" may be used if the product contains 3 g or less of fat per 240 mL (1 cup) which is considered the reference amount. To be labeled "fat free," "free of fat," "no fat," "zero fat," "without fat," "negligible source of fat," or "dietarily insignificant source of fat," the milk must contain less than 0.5 g fat per 240 mL (1 cup). In the case of milk, an additional term for this "fat free" category is "skim."

With regard to fat, flavored milk and specialty products must meet the same labeling requirements as plain milk products. Milk may be flavored with a wide variety of products including but not limited to vanilla, chocolate, coffee, mocha, and fruit essences and juices. An example would be "low-fat chocolate milk." Eggnog is described in Title 21 CFR Part 131.170 (USHHS-FDA, 2007a) as a food containing specific cream or milk products, one of more egg yolk-containing products and a sweetener. The formulation must result in a product with not less than 6% milk fat, not less than 8.25% MSNF, and not less than 1% egg yolk solids. As with other fluid milk products, eggnog may have modified fat content according to NLEA standards.

The final category of fluid milk products is those containing significantly higher amounts of milk fat than milk and is known as "cream." One of the two primary products of separation processes, "heavy" cream is described in 21CFR131.150 as a product containing not less than 36% milk fat. "Light" cream must contain not less than 18% and less than 30% milk fat (USHHS-FDA, 2007a). "Light whipping" cream must contain not less than 30% but less than 36% milk fat (USHHS-FDA, 2007a). The final cream product identified in the CFR is "half-and-half" which is a mixture of milk and cream containing not less than 10.5% but less than 18% milk fat (USHHS-FDA, 2007a). All cream products may also have safe and suitable optional ingredients including emulsifiers, stabilizers, nutritive sweeteners, colors, and flavors added for functional purposes.

A subcategory of fluid milk products that is gaining in importance is “organic” fluid milk and cream products. Products sold under the organic label must meet the USDA National Organic Program (NOP) standards for production of organic foods including milk (USDA, 2007c). Retail sales of organic milk products have steadily grown since the mid-1990s, attaining a 6% share of the fluid milk market in 2006 (Dimitri and Venezia, 2007). The majority of organic milk in the United States is collected and processed by three large companies necessitating ultra-pasteurization to extend the shelf life of the product. The extended shelf life allows for the distribution of the organic milk throughout the conventional and health food marketing channels.

Finally, all the above products should be provided a heat treatment, such as pasteurization, or other process to help ensure the safety of the final product. The following discussion will demonstrate how the variety of fluid milk products discussed above can be processed within a system that can provide flexibility to handle all the compositional, quality, and safety requirements for fluid milk products.

RECEIVING

Upon arrival at a transfer station, receiving station or processing facility, preparation for the transfer of milk from the bulk milk tanker to the storage tank will include procedures for inventory, safety, security, and quality. Although the scale of the facility receiving the milk will result in variations in methods, the functions will all be performed to at least a minimal/legal level.

Inventory of milk in small plants or transfer stations may be as simple as summing the measurements on milk hauler’s individual producer receipts. In this case, the inventory of milk being received is dependent on the quality of work being done by the bulk milk hauler and special care should be taken to ensure accurate readings by the hauler and accurate bulk tank calibration charts. In many cases, the first activity upon arrival at the processing facility will be the weighing of the loaded tanker. The inventory function is then completed by weighing the empty tanker upon completion of receiving. Metering milk is also an acceptable procedure when using an appropriately designed metering system. During the pumping operation the beginning and end of the milk flow is often accompanied by substantial amounts of air, therefore, the metering system must be designed to accommodate the problems associated with a two-phase prod-

uct flow. A fourth method involves pumping the milk into tanks mounted on load cells. Although an acceptable and accurate system for inventory, pumping the milk into and out of intermediate, load cell-equipped tanks will result in extra time and handling of the milk. Since each occurrence of pumping or agitation may result in degradation of the fat globule membrane and subsequent development of hydrolytic rancidity, the load cell method should be used with great care.

The samples attained by the hauler from each producer’s bulk tank before being pumped onto the truck should be checked for appropriate temperature and immediately placed in an appropriate refrigerator for future testing. The individual producer samples will be used to determine composition, bacteria count, and somatic cell counts which when combined with weights from hauler receipts will determine the paycheck for the producer. Before any milk may be pumped off the farm pick-up tanker, the receiving technician should check to make sure the wash tags and security seals are appropriate before a representative sample is taken. All samples must be taken from a properly mixed tanker, therefore, mechanical or air agitation of the tanker may be required if a period of quiescence has occurred after the arrival of the tanker at the receiving facility. The receiver will take the required sample and measure the temperature from the access port on the top of the tanker. The temperature of the milk in the tanker must be less than 45°F (7.2°C) to be legally acceptable, however, most processors of fluid milk will require the milk to arrive at the plant at less than 40°F (4.4°C). The receiving technician should take a deep breath through his/her nose to determine if the milk has an acceptable odor. Any off-odors should be noted and reported to supervisors before unloading the tank.

For milk safety, all milk for fluid consumption must be screened for antibiotics before being pumped from the farm pick-up tanker to storage tanks or larger tankers where commingling with milk from other farm pick-up tankers may occur. The procedures for testing for antibiotic residues in milk are found in Appendix N of the PMO. Once the screening indicates a negative result for antibiotic residues the milk is legally approved for transfer to storage tanks. If the milk is being transferred to a larger tanker for transport, no further testing of the milk will be necessary at the final destination. If there is a positive result for antibiotic residues, confirmatory tests must be run on the tanker sample and the individual producer samples from that load to determine the source of the contamination.

Prior to unloading, milk being received at the processing facility will likely be screened for titratable acidity (TA), a test that will indicate a poor temperature history by quantifying any lactic acid production, and for total bacteria by the Direct Microscopic Count (DMC) method (Wehr and Frank, 2004). A titratable acidity above 0.18% and/or a DMC of 100,000 colony-forming units (CFU) per mL or g will result in rejection of a load of milk at a fluid milk facility. The legal commingled bacteria count at the dairy plant according to the PMO is 300,000 CFU/mL; however, this is considered too high to produce high-quality fluid milk products. Loads of milk received at dairy plants today often have total bacteria counts less than 10,000 CFU/mL. The load sample or a duplicate will be retained for further testing. After the milk is unloaded the samples may also be tested for freezing point, bacteria, and composition. The composition test will be important to determine the inventory of milk components in the raw storage tanks.

Once the receiving tests have been completed, usually a 15-minute task, the milk may be pumped into the storage tanks. Vents must be open prior to pumping off the tanker to avoid creating a vacuum resulting in collapse damage to the tank. If the receiving hose and the pick-up hose are separate, the pick-up hose and the pick-up pump may be cleaned while the milk is being pumped off the truck. However, no cleaning procedures on the outside of the truck should be initiated until all the milk has been removed from the tank. This will prevent contamination of the milk in the tank. Once the milk is out of the tanker, the technician will attach the clean-in-place system and initiate the cleaning cycle. At this time, the hauler may clean the exterior surfaces of the tanker. Once the appropriate rinse and clean cycles have been completed the receiving technician will attach a cleaning tag noting the truck ID, location of the cleaning, time, date, and signature or initials of the technician responsible for cleaning. Measures to ensure the safety and security of the milk products including the attachment of a security seal are important at all stages of transfer and transport. Dairy Practices Council (DPC, 2005) and FDA (USHHS-FDA, 2003b) guidelines for security and safety provide ample guidance in this area.

RAW STORAGE

Milk may be stored in any vessel that meets the requirements of the PMO. Legally, the temperature must be maintained below 45°F (7.2°C) for the duration of the storage period which cannot be more

than 72 hours. Fluid milk processors should make every effort to maintain temperatures below 40°F (4.4°C) and to move product out of storage within 24 hours whenever possible. Storage tanks used for storage longer than 24 hours must be equipped with a 72-hour temperature recording chart. Maintaining proper temperatures may be done with insulated tanks for storage periods of 24 hours or less; however, longer storage periods will require cold wall tanks using either direct expansion or circulation of a refrigerated fluid such as ice water or glycol. Because of the natural separation (creaming) of raw milk, storage tanks must be equipped with agitation capabilities. A well-mixed raw milk supply will allow for proper processing and standardization of finished products.

To reduce the possibilities for cross-contamination of finished product by raw product, the personnel involved in the hauling and receiving of raw milk should not be allowed into the finished product areas of the fluid milk processing plant. The air handling facilities should also be designed to prevent movement of air from the raw milk to the finished product areas of the plant.

The four major unit operations that take place between storage of raw milk and storage of finished product prior to packaging are (1) separation, (2) standardization, (3) heat treatment, and (4) homogenization. These unit operations may be combined in a wide variety of configuration and are not all required for each individual fluid milk product. The common denominator for all fluid milk operations is proper sanitary design and operation of the equipment and facility to maintain maximum allowable shelf life for the finished products.

SEPARATION

The native milk fat globule ranges in size from 3 to 20 μm resulting in an oil-in-water emulsion that will naturally separate into skim milk and cream under the influence of gravity. The speed of separation of a perfect sphere is explained by the Stokes equation. Since milk fat globules are not necessarily perfect spheres, these become a good approximation and will vary with milk from different sources exhibiting variable physical and rheological characteristics.

$$v_t = D^2(\rho_L - \rho_P)g/18\eta_L,$$

where v_t is the terminal velocity; D is diameter of particle; ρ_L is mass density of liquid phase; ρ_P is mass density of particle; g is acceleration due to gravity; and η_L is viscosity of liquid phase.

The velocity of the particle (milk fat globule) will increase as the diameter of the particle and the difference in density between the liquid and particle phases increase, while the velocity will decrease as the viscosity of the liquid phase increases. Raw milk contains an immunoglobulin (IgM) that facilitates the agglutination (clustering) of the globules which effectively increases the diameter of the particle. Therefore, cold raw milk will demonstrate the creaming phenomenon at a faster rate than would be predicted by the diameter of the milk fat globule alone. Heating milk to pasteurization temperature and time standards will destroy the cold agglutination phenomenon.

Using this natural separation, man developed a variety of milk and milk by-products with varying fat composition; however, the time required for gravity separation and the difficulty in isolating the two phases at a consistent composition did not allow for efficient production of consistent, quality products. The introduction of centrifugal separation technology has accelerated the process allowing rapid isolation of skim milk and cream phases with consistent and controllable compositions.

Centrifugal separation is accomplished by substituting a rotational component for the gravitational acceleration (g). The effective radius of the centrifuge (R) multiplied by the square of the angular velocity (ω^2) results in a new equation that represents the velocity of separation in the centrifugal environment ($v_s = D^2(\rho_L - \rho_P)R\omega^2/18\eta_L$). The fat globules will accelerate toward the center of the centrifuge bowl and are aided in separation by a stack of discs that create a short distance for separation (Fig. 9.1).

Centrifugal separation may be done when the milk is either cold or hot depending on the requirements of the processor. Heating milk to a temperature of 50–60°C (122–140°F) just before separation will (1) melt all of the fat crystals, (2) increase the difference in density of skim milk and milk fat, (3) increase the size of the milk fat globule, and (4) decrease viscosity of the skim milk resulting in skim milk with 0.01–0.02% milk fat and cream ranging from 20 to 70% milk fat. The fat content of the cream is controlled by adjusting the amount of skim milk forced to exit through the cream outlet with the milk fat globules. A hot milk separator will generally be included in the flow of a continuous heat treatment system and may be on the raw side or the finished side of said system.

When separated cold, the skim milk will be slightly higher in fat (0.1–0.2%), resulting in a product that many consumers consider superior due to a slightly more opaque, white color, and mouthfeel. Because of

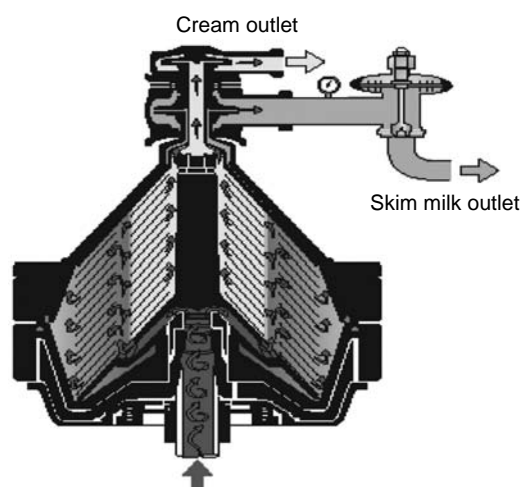


Figure 9.1. Hermetic separator bowl with an automatic pressure unit on the skim milk outlet. (Reproduced by permission of Tetra Pak, Lund, Sweden.)

the partial crystallization of the milk fat, the cream will be limited to a maximum of about 45% milk fat due to the viscosity of the cold cream phase. Cold milk separators require more internal space to allow for flow of the viscous cream phase, thus are not interchangeable with warm milk separators.

Particles such as dirt, somatic cells, and some large casein micelles are denser than the liquid phase. Therefore, acceleration toward the outside wall of the separator bowl will occur. These components will collect as “sludge” at the wall of the separator bowl. The sludge may be removed manually at the time of cleaning in older, small separators. Periodically modern, self-desludging separators will hydraulically open at the sidewall to expel the sludge using centrifugal force, thus allowing for extended operation and cleaning in place.

STANDARDIZATION

Once the milk has been separated the resulting products can be combined in a variety of process systems to make products with standardized milk fat contents. The standardization system may be either batch, continuous flow or a combination operation. A typical batch operation would simply involve sending the skim milk and cream from the separator to storage tanks which could be drawn upon for required quantities for standardized products (Fig. 9.2). The

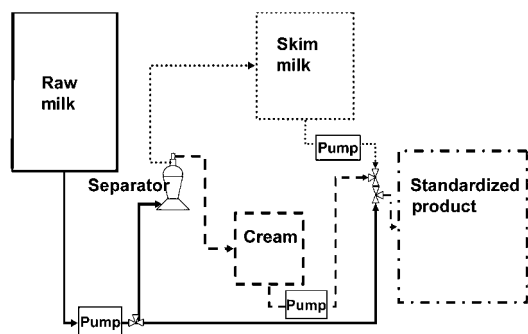


Figure 9.2. Schematic of Batch Standardization System.

skim milk and cream may also be combined with other products such as whole milk to meet the target composition (see Table 9.1). The only requirement is that the two products have near-equivalent nonfat solids content in the aqueous phase and the fat contents bracket the standardized product. Appropriate portions of two products bracketing the fat content of the target product may be added to a third tank to make the standardized product. A semicontinuous operation using metering technology would allow the two products to be combined in stream thereby eliminating one tank from the facility.

The capability for rapid in-line analysis of the cream phase, constant composition of the skim milk phase, and accurate metering technology allows for the adjustment of milk fat percentage on a continuous basis (Fig. 9.3).

When standardizing to a milk fat level lower than the original milk supply, the continuous standardization system will result in a stream of excess cream along with the standardized milk.

Standardization for MSNF is also practiced by some plants for specific fortified products, and requires a little more complicated calculation for the standardization math due to the inclusion of three ingredients. The standardization simply requires the algebraic solution of three simultaneous equations. The equations consist of (1) total weight, (2) fat content, and (3) MSNF content of the three ingredients. Generally, the ingredients for this process are two products for standardization of the milk fat content and one concentrated source of MSNF such as Grade A nonfat dry milk powder or condensed skim milk. After the standardization has taken place the product

Table 9.1. Standardization Examples

Unlimited Available Products:

- Skim milk (0.1% milk fat)
- Whole milk (3.7% milk fat)
- Cream (40.0% milk fat)

Desired Products and Potential Ingredients:

100,000# Low-fat milk (1.0% milk fat)

Solution A for low-fat:

Combine skim milk and cream

Solution B for low-fat:

Combine skim milk and whole milk

20,000# Light cream (18.0% milk-fat)

Solution A for light cream:

Combine skim milk and cream

Solution B for light cream:

Combine whole milk and cream

Example Calculations for Solution A for Low-fat:

x = lb. of 40% cream

y = lb. of 0.1% skim milk

Solve simultaneous equations

where x = lb. of cream and y = lb. of skim milk

(1) $x + y = 100,000$ lb. of low-fat

$\Rightarrow x = 100,000 - y$

(2) $0.4x + 0.001y = 0.01$ (100,000 lb. of low-fat)

(3) $0.4(100,000 - y) + 0.001y = 0.01(100,000)$

(4) $40,000 - 0.4y + 0.001y = 1,000$

(5) $40,000 - 1,000 = 0.4y - 0.001y$

(6) $39,000 = 0.399y$

(7) $y = 97,744.36$ 0.1% skim needed

(8) $x = 100,000 - 97,744.36 = 2,255.64$ lb. of cream needed

Check:

$2,255.64 \text{ lb.} \times 0.4 \text{ lb. fat in 1 lb. cream} = 902.26 \text{ lb. fat}$

$97,744.36 \text{ lb.} \times 0.001 \text{ lb. fat in 1 lb. skim} = 97.74 \text{ lb. fat}$

$100,000.00 \text{ lb.} \times 0.01 \text{ lb. fat in low-fat} = 1,000.00 \text{ lb. fat}$

is ready for further processing, which may or may not include vitamin supplementation, homogenization, and/or heat treatment.

VITAMIN SUPPLEMENTATION

As indicated in the descriptions of fluid milk products, vitamins A and D may be either mandatory or optional additions. Because of the fat-soluble nature of vitamins A and D, fortification must take place after separation/standardization operations. Addition of supplements must also be prior to pasteurization. Vitamins may be added as a batch operation to a definite quantity of milk collected in a storage

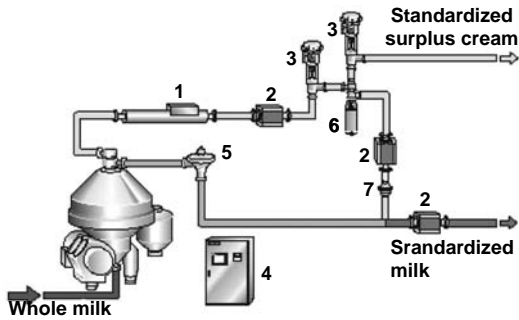


Figure 9.3. The complete process for in-line standardization of milk and cream. (1) Density transmitter, (2) Flow transmitter, (3) Control valve, (4) Control panel, (5) Constant pressure valve, (6) Shut off valve, and (7) Check valve. (Reproduced by permission of Tetra Pak, Lund, Sweden.)

vessel or may be added on a continuous basis using a sanitary, positive-displacement pump to add to the constant level tank or to inject into the pipeline. When adding vitamins on a continuous basis the pump must be wired to allow operation only when the pasteurization system is in forward flow (see Continuous Pasteurization section below). Vitamin addition must be done very carefully to ensure the customer gets the proper amount and no overdosing occurs.

HOMOGENIZATION

The first homogenization of milk is attributed to Augustus Gaulin of France in 1899 and was followed by his patent of 1902 which described a homogenizer operating fundamentally the same as modern homogenizers. Homogenization is accomplished by pumping the milk through a small aperture at a pressure of 103.4–124.1 bar (1,500–1,800 psi). The milk is accelerated to a high velocity and impacted on a surface perpendicular to the flow of the product, thus shattering the milk fat globules (Fig. 9.4). The milk fat must be in a liquid state for efficient homogenization, therefore the temperature of the milk must be at least 37°C (99°F). Other physical forces such as attenuation, cavitation, and shearing may also contribute to the size reduction. The milk fat globules will be reduced from 3 to 20 µm to less than 2 µm in diameter. Then, as now, the goal was to reduce the size of the fat globule to such a diameter that they

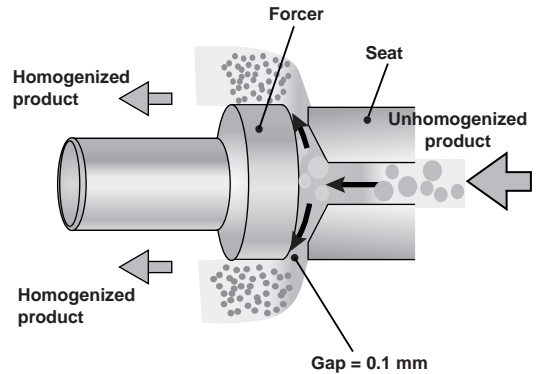


Figure 9.4. The milk is forced through a narrow gap which results in the fat globules splitting into smaller sized droplets. (Reproduced by permission of Tetra Pak, Lund, Sweden.)

can no longer rise in the aqueous phase resulting in a stable, homogeneous product with no cream layer forming during storage. After 48 hours of quiescent storage at 4.4°C, the top 100 mL of one quart of homogenized milk or other milk product will not differ in fat percentage by more than 10% from the remaining, thoroughly mixed sample.

The serum phase of milk contains a lipoprotein lipase that will split apart the triglycerides of milk fat if the milk fat globule membrane is damaged. Since the function of homogenization results in globule disruption and greatly increased surface area, homogenization must take place following heat treatment or immediately prior to heat treatment in a continuous flow process system. The heat treatment will inactivate the lipase enzyme thus avoiding hydrolytic rancidity caused by release of volatile fatty acids. The mixture of raw milk with heat treated, homogenized milk will also result in rancid products.

HEAT TREATMENT

The consumption of raw milk has been a part of our world since the first use of milk from other species by man; however, there are substantial, scientific arguments for prohibiting the sale of raw milk to the consuming public. Milk is produced in an environment that is inherently capable of harboring pathogenic (disease causing) bacteria that can contaminate the milk during the milk harvesting process. Despite the conscientious efforts of milk producers to avoid

contamination by bacteria, a small number will always be present in the milk. Because of the small number of bacteria considered an infectious dose for some pathogenic species, 15–20 cells for *Salmonella* spp. (USHHS-FDA, 2006), the consumption of raw milk is a practice that carries more risk than most people should be willing to assume. The distribution of raw milk is not legal for interstate commerce. There are several states that allow the sale of raw milk under certain circumstances such as at the farm only. The arguments around the consumption of raw milk are complex and will not receive further discussion in this volume.

The application of heat to milk for fluid consumption has the following basic functions: (1) the elimination of pathogenic (disease causing) bacteria; (2) the reduction or elimination of spoilage bacteria; and (3) the inactivation of select indigenous enzymes. Since all the legally acceptable heat treatment methods for fluid products will perform the functions of pathogen elimination and lipase inactivation, the selection of heat treatment method is typically based on the need for shelf life and flavor characteristics. The major classification of heat treatments include pasteurization and sterilization. Pasteurization procedures will not eliminate all spoilage bacteria and therefore must be refrigerated throughout their shelf life. Sterilization procedures will eliminate all bacteria and inactivate the majority of enzymes in the milk, allowing the potential for ambient temperature storage if handled and packaged in an aseptic system after heat treatment.

BATCH PASTEURIZATION

Batch pasteurization procedures were the first instituted in the late 1800s by pioneers of heat treatment, including Louis Pasteur, 1867, and Franz von Soxhlet, 1891. The current time/temperature relationship for batch pasteurization of milk is 63°C (145°F) for 30 minutes and is often referred to as low temperature, long time (LTLT). If a fluid milk product such as chocolate milk or cream contains 10% or more milk fat or contains added sweeteners, the temperature must be increased by 3°C (5°F). Eggnog must be processed at 69°C (155°F) for 30 minutes. Batch pasteurization equipment is relatively simple and provides flexibility with regard to product variety.

A vessel is required to have a cover with agitator, product, and instrumentation openings that properly shield the milk from outside contamination such as

splash, airborne dust, and condensation, all of which may carry disease causing bacteria or other hazardous contaminants. The heating and cooling of the product may be done in the vessel using appropriate heating or cooling media circulated through the wall or floor of the vessel. Often times the cooling is done by external continuous heat exchangers such as a plate or tubular system. The 30-minute holding period may not start until the entire product in the vessel is at the target temperature and no product may be removed before the holding period is complete. Because of their flexibility, batch pasteurizers are often used by small processors or for small batches of products in large plants.

The vessel used for batch pasteurization must be designed to provide adequate agitation of the product throughout the holding period to maintain the required temperature and allow no more than a 0.5°C (1°F) difference between the coldest spot in the vessel and any other portion of the product when measured simultaneously. The pasteurization vessel must be equipped with an indicating thermometer, a recording thermometer, and an air space thermometer (Fig. 9.5; USHHS-FDA, 1998). The indicating thermometer allows the employee responsible for the process to visually verify the temperature and note said temperature on the recording thermometer chart at the

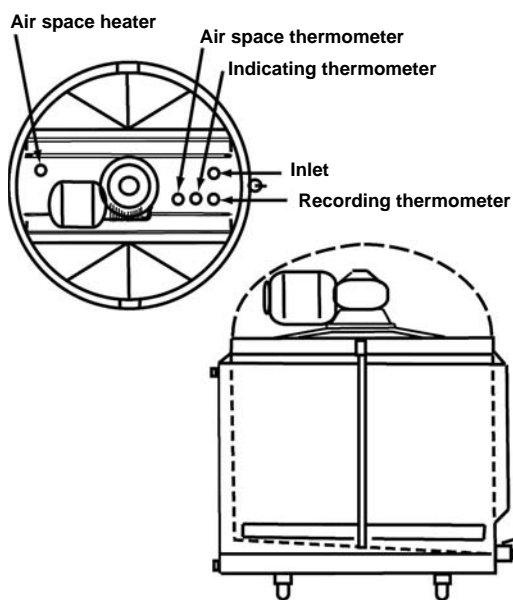


Figure 9.5. Batch pasteurizer (USHHS-FDA, 1998).

beginning of the 30-minute holding period. The thermometers must be checked at each regulatory inspection and the indicating thermometer may never read lower than the recording thermometer.

The air space thermometer is required to measure the temperature of the air space above the product at the beginning and the end of the holding period. These temperature readings must then be recorded on the recording thermometer chart. The air above the product is normally heated with culinary steam but may be heated by a solid-state heating element compatible with the sanitary operation of the pasteurization vessel.

The batch pasteurization vessel must also be equipped with a close-coupled, leak-detect outlet valve. The close-coupled design must prevent milk in the valve inlet from varying by more than 0.5°C (1°F) from the center of the vessel. The leak-detect design prevents the leakage of milk past the fully closed outlet valve. The outlet must remain fully closed throughout filling, heating, and holding periods. For a detailed description of the sanitary standards for a batch pasteurizer one should refer to *The 3-A Sanitary Standards for Non-Coil Type Batch Pasteurizers For Milk and Milk Products* (3-A SSI, 1989).

CONTINUOUS PASTEURIZATION

The fact that time required for destruction of bacteria and inactivation of enzymes decreases logarithmically as the temperature of processing increases has allowed the design of continuous flow pasteurization systems. Table 9.2 contains the acceptable minimum temperature/time relationships for pasteurization of milk and milk products.

The most frequently used temperature/time relationship for pasteurization is 72°C (161°F) for 15 seconds. The holding time of 15 seconds is easily accomplished by pumping the product through a length of tubing at a specific flow rate. The temperature and times are minimums and may be exceeded and generally are for the purposes of processing fluid milk products. These continuous systems are referred to as "high-temperature, short-time" (HTST) pasteurizers. Depending on the location of separation, standardization, and homogenization equipment, the configuration of the HTST systems may vary in design and complexity. The components and design of a basic HTST pasteurization system are illustrated in Figure 9.6. Although the plate heat exchanger is the most common, other heat exchange units such as swept surface and tubular designs may also be used.

Table 9.2. Pasteurization Temperature Versus Time

Temperature	Time
*63°C (145°F)	30 minutes
72°C (161°F)	15 seconds
89°C (191°F)	1.0 seconds
90°C (194°F)	0.5 seconds
94°C (201°F)	0.1 seconds
96°C (204°F)	0.05 seconds
100°C (212°F)	0.01 seconds

*If the fat content of the milk product is 10% or more, or if it contains added sweeteners, the specific temperature shall be increased by 3°C (5°F). *Provided*, that eggnog shall be heated to at least the following temperature and time specifications:

69°C (155°F)	30 minutes
80°C (175°F)	25 seconds
83°C (180°F)	15 seconds

Provided further, that nothing shall be construed as barring any other pasteurization process which has been recognized by the Food and Drug Administration to be equally efficient and which is approved by the regulatory agency

Source: USPHS-PMO.

In the basic system illustrated in Figure 9.6, raw milk is pumped from storage to the constant level tank, which ensures that no air enters the HTST system. The product is then drawn through the regeneration section where the cold milk at 5.6°C (42°F) flows on one side of the plates in the heat exchanger and hot pasteurized milk at 72.2°C (162°F) flows on the opposite side. The counter-current flow illustrated in Figure 9.7 will result in efficient reclamation of heat, making the HTST system much more energy conservative than the batch pasteurizer which requires external energy inputs for the entire heating process. Temperature at the outlet of the regeneration section will be 65.6°C (150°F) in a system designed for 90% regeneration.

The next unit in the pasteurization system is the timing pump. In the basic system, a positive displacement pump is used to establish the proper flow rate through the pasteurizer. The use of centrifugal pumps is permitted with the addition of flow meters and a flow controller to adjust the speed of pumps and position of valves used to promote and control product flow. Meter-based systems are very effectively used in larger dairy operations.

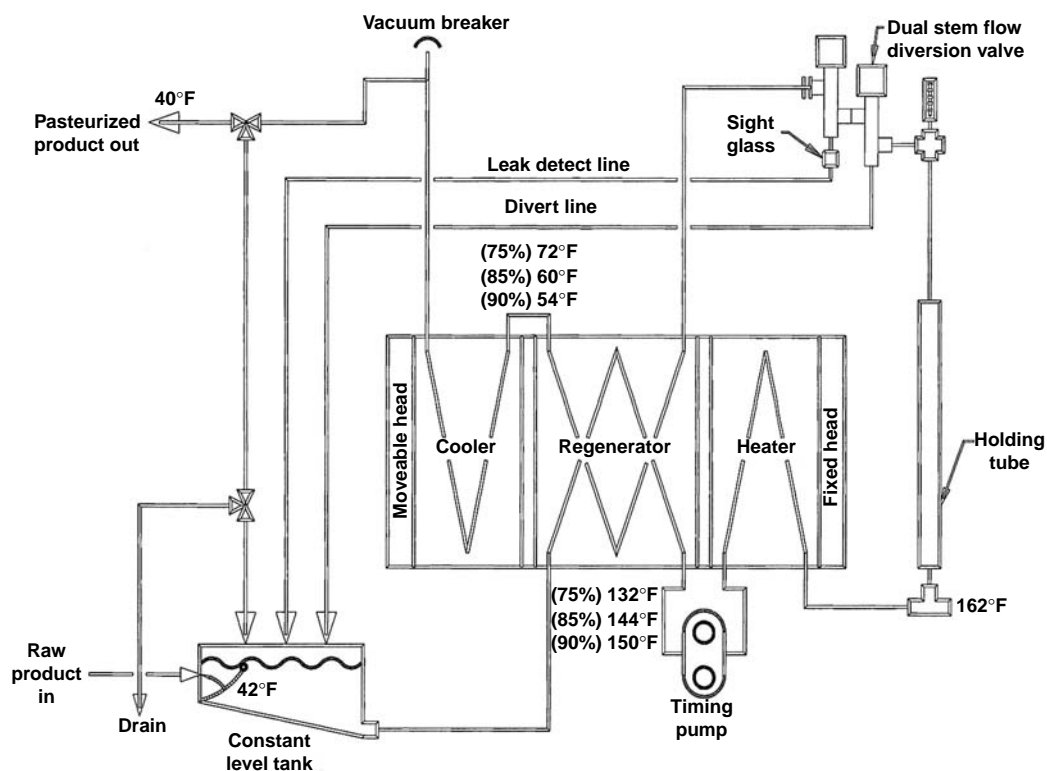


Figure 9.6. Basic high-temperature, short-time (HTST) pasteurization system with temperature progression for 75%, 85%, and 90% efficient regeneration section. (Reproduced by permission of Seiberling Associates, Inc., Dublin, OH.)

The product is heated in the heater section and upon exiting, enters the holding tube. The maximum allowable flow rate, as determined by conductivity testing of the system in operation with water, is that which results in at least the minimum legal time of 15 seconds in the holding tube. The regulatory inspector seals the timing pump or the flow controller in such a manner as to prevent operation at a flow rate higher than the maximum allowed. Practically speaking, all pasteurizers will have a holding time of 16 seconds or greater due to the limitations of regulatory testing and system control instrumentation. Upon reaching the end of the holding tube, the product will pass the recording and indicating thermometers followed by the flow diversion device (FDD).

If the product reaches the recording thermometer below the legally allowable temperature, a signal will be sent from the recorder-controller and the flow diversion device will go into the diverted flow position

and return the unpasteurized product to the constant level tank for reprocessing. A few single stem (valve) FDDs may still be found in smaller dairy plants, however, most plants are equipped with dual-stem FDDs. The first valve in the dual-stem FDD is the flow diversion valve while the second valve acts as a leak detection device to make sure that no diverted product is getting past the flow diversion valve into the pasteurized side of the system. As in the batch pasteurizer, both indicating and recording thermometers are required. Each time the HTST pasteurizer operator starts the system, a check of the cut-in temperature that initiates forward flow and cut-out temperature that initiates diverted flow must be recorded on the recording chart.

In a meter-based system, the FDD will also go into diverted flow if the flow rate increases to a level that reduces the holding time below that required for pasteurization of the product. A third control mechanism

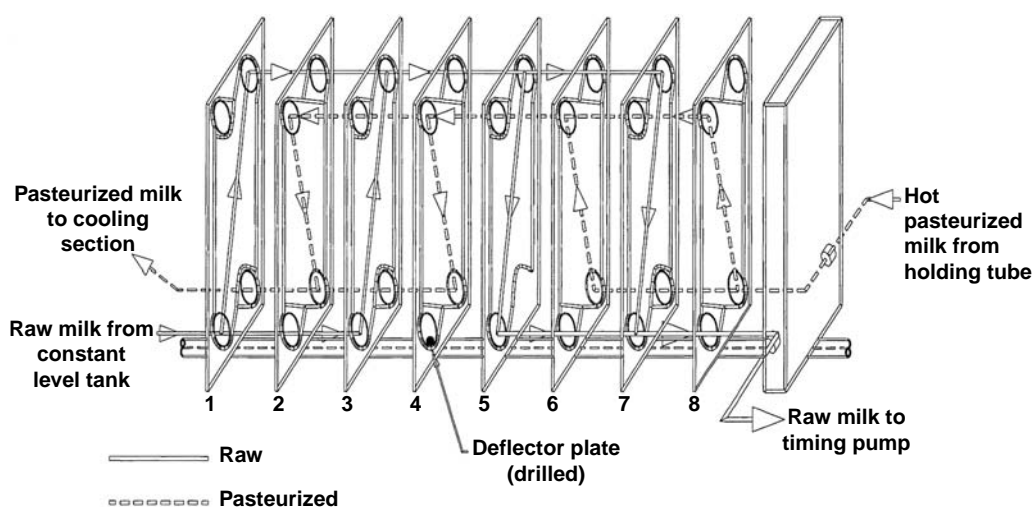


Figure 9.7. Countercurrent flow through simple two pass, two stream regeneration section. (Reproduced by permission of Seiberling Associates, Inc., Dublin, OH.)

may be found in the pressure differential switch. To avoid contamination of pasteurized milk by raw milk in the regeneration section of a booster pump equipped system pressure sensors are placed at the raw milk inlet and the pasteurized milk outlet of the section. These sensors are then connected to a pressure differential switch that continually monitors the system to ensure that the pasteurized side of the regenerator is always at a higher pressure than the raw side. If the pressure differential is not adequate at any time in the process the booster pump will stop. All diversions will continue until the abnormal condition has been corrected.

If all control conditions are met the product will continue by going back through the pasteurized side of the regeneration section where a 90% efficient system will reduce the temperature to 12.2°C (54°F). The last section of the heat exchanger is the cooling section, where ice water or glycol will be used to reduce the temperature to less than 4.4°C (40°F). After cooling the product is stored in an appropriate vessel and is ready for packaging.

Many modifications to the basic HTST pasteurization systems are possible (3-A SSI, 2005). The efficiencies of both separation and homogenization are increased when the product is warm therefore inclusion of these process units within the HTST system is common. The regeneration section may be split to allow either warmed raw or partially cooled pas-

teurized milk to be diverted to a separator or homogenizer at 50–60°C (122–140°F). Homogenization is often done between the regeneration and heating sections as well. If either of these operations is placed on the pasteurized side of the system there may be an increased chance for postpasteurization contamination, therefore the recommended practice is to keep these processes on the raw side of the system.

Similar systems that use temperatures above 89°C (191°F) and reduced holding times of 1 second or less are referred to as “higher-heat, shorter-time” (HHST). A significant modification for an HHST system is the placement of the FDD after either the final regeneration or cooling sections of the heat exchanger to allow for reaction time in case of a process deviation. The higher temperatures of these systems allow for increased bacterial kill and inactivation of enzymes leading to a longer shelf life while still maintaining much of the flavor profile of pasteurized milk. They are often referred to as “extended shelf life” or ESL products. If the product is heated to at least 138°C (280°F) for at least 2 seconds the label will indicate the product has been “ultra-pasteurized (UP).”

These systems may include common indirect heating methods which utilize heat exchangers with a stainless steel interface between the product and the exchange media or direct systems which either inject or infuse culinary steam directly into the product. The injection system uses an in-line nozzle to inject the

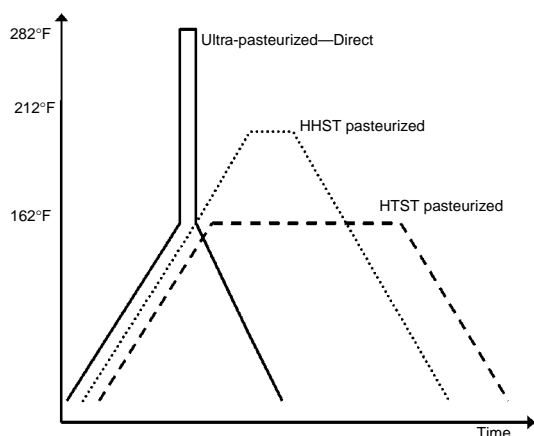


Figure 9.8. Temperature profiles for pasteurization processes. (Adapted from *Technology Update*, APV Fluid Handling, Lake Mills, WI).

steam directly into the product while the infusion system requires a large steam chamber where milk falls through the steam. The use of direct heating systems has the distinct advantage of doing much less damage to the components of milk that lead to potential color and flavor changes. The reason for this can be seen in Figure 9.8 which shows the temperature profile for three types of pasteurization systems. The almost instantaneous increase and decrease in temperature on the ultra-pasteurization curve are a result of direct contact of steam with the product followed by treatment in a vacuum chamber to rapidly cool the product by evaporation of the water added during the injection/infusion. The indirect systems require more heating and cooling time resulting in significant increases of cooked flavors in ESL products from indirect heating HHST systems.

The combination of dairy plant consolidation, extended distribution systems, and the need to get small volume of niche products to the consumer in a wholesome, safe manner has opened the door for expansion of the ESL market. The combination of UP processing, sterile packages, sanitary fillers and refrigeration yields fluid milk products with shelf lives as long as 90 days compared to 10–28 days for conventionally pasteurized products. An example of small volume products would be cream products that were short shelf-life products until ultra-pasteurization was introduced and processing was centralized. The current market for organic milk is

a prime example of the use of ESL technology to facilitate the national distribution of a niche product from a relatively small number of producers and processors.

STERILIZATION

A further step in the heat treatment of milk products uses technology similar to ultra-pasteurization systems. The ultra-high temperature (UHT) sterilization of milk is most effectively achieved with direct steam injection or infusion. While ultra-pasteurized (ESL) products require cooling to temperatures less than 4.4°C (40°F) prior to packaging and storage, sterile UHT products need only be cooled to room temperature prior to packaging the product.

The regulations and guidelines for aseptic (UHT) processing of milk may be found in Title 21 CFR Sections 108 and 113 rather than the PMO. These products are treated the same as low-acid canned foods. The process used, which is typically 146°C (295°F) for 4 seconds, must be filed with FDA by the Process Authority sharing responsibility for the plant. The higher temperature–time relationship ensures a commercially sterile product. The sterile product is then handled in an aseptic manner to prevent any postprocess contamination. Pipelines, valves, and holding tanks are designed with steam seals to prevent any contamination. The product must also be filled into a sterile container in a sterile (not just sanitary) environment. These requirements result in much great capital outlay for equipment and installation of aseptic systems. However, the product can be stored for a minimum of 6 months at room temperature which presents a large savings in refrigeration expense.

PACKAGING

The evolution of packaging for fluid milk products has resulted in a diverse offering of options that fit the needs of a diverse population. Packaging can be broken down into single-use (disposable) or multiuse (refillable) containers. Glass bottles are the most common multiuse container. Though they have appealed to those who are concerned about disposables clogging our landfills they have some inherent problems that make them a less desirable container for most markets. Glass bottles are heavy, fragile, and bulky and when returned to the dairy plant for cleaning and refilling they have potential for bringing contaminants back into the plant environment. Several

good alternatives to multiuse containers have been developed over the past 70 years.

Milk products are currently packaged in a wide variety of paperboard, plastic and composite materials for both the retail and wholesale markets. For a detailed discussion of packaging of dairy foods see Chapter 20.

One unit operation that has become increasingly important in the dairy industry is the coding of products. Some states require a sell-by date while others require an expiration date. Coding systems are available that can add valuable data including the time of day and the packaging equipment used. No matter how small or large a plant is, there should be a method of coding products to allow for identification for quality assurance and for possible recall of a product not meeting the quality or safety parameters of the law or the company.

PRODUCT SAFETY AND QUALITY ASSURANCE

Although the most obvious point in the fluid milk processing system for safeguarding the consumer is the heat treatment system, there are many other areas that contribute to the production of safe, wholesome milk products. Assuming that raw milk and other ingredients are of high quality when they reach the fluid milk plant, the job at hand is to enhance the safety and preserve the quality as the products progress through formulation, processing, packaging, and distribution to the consumer's custody. A foundation to build on in any food environment is the use of current good manufacturing practices (GMPs), which are published in Title 21 CFR Part 110 (USHHS-FDA, 2003c). The Grade A PMO incorporates GMPs throughout the document but a good management team will be familiar with the provisions of both. Conscientious incorporation of GMPs in to the specific daily activities of the plant through proper training of personnel will result in few opportunities for contamination of the products in the custody of the plant.

The Grade A Pasteurized Milk Ordinance lays out the requirements for enhancing the safety of fluid milk products by the elimination of pathogenic bacteria. Once the product has been pasteurized the product must be protected from post-pasteurization contamination. Therefore, the cleaning and sanitation programs for equipment and environment must be adhered to and supported by the entire employee pool from labor through top management. Inspection by the state regulatory agency will occur at least four

times per year and samples are typically taken on a monthly basis for evaluation of microbiological and chemical parameters. An alternative to the normal PMO-based inspection system is the *Dairy Grade A Voluntary HACCP* (Hazard Analysis and Critical Control Point) program (USHHS-FDA, 2007c). This program requires both the plant and the regulatory agency to commit to the HACCP program and can result in semiannual inspections once the program is running to the satisfaction of the regulatory agency. All standards for the products and plants must meet at least the same levels as indicated in the Grade A PMO.

Regular chemical and microbiological testing of products, process equipment, and the plant environment yields valuable information for the maintenance of quality and safety of the fluid milk products; however, collection of data without planned and implemented analysis of said data will not be useful. Statistical quality assurance programs should be evaluated on a regular basis to look for hot spots and trends that may lead to improvement of quality and safety of products. Observation of trends is especially important in a fluid milk operation because often the product is in the distribution channel before the results of the tests become available.

COMPOSITIONAL EVALUATION

The process facility should have a laboratory capable of performing appropriate compositional tests. Using an outside laboratory for this purpose will not provide the timely results needed due to the rapid turnover of product in inventory of fluid milk operations. The products must meet the legal minimum fat and milk MSNF for each product that is processed in the facility. To perform proper standardization of the products the components of the inputs must be known prior to calculation of input ratios. Milk fat may be determined by a variety of chemical methods including the Babcock or Gerber volumetric tests (sulfuric acid digestion) or the Roesse-Gottlieb or Mojonnier gravimetric tests (ether extraction). Milk solids not fat are typically determined by analyzing for total solids and subtracting the milk fat content. Total solids are determined by drying a given sample by vacuum oven, microwave or infrared methods. All the above methods will provide sound data if run by a competent laboratory technician. In large plants, the need for rapid compositional test results has encouraged the development of instrumental methods that can provide single- or multicomponent analysis

in a few seconds or minutes. Utilizing technology based on turbidity or ultrasonic, infrared (IR), mid-infrared (MIR), or near infrared (NIR) spectroscopy test results for milk fat, total solids, MSNF, protein and/or lactose are available for immediate use in formulation and release for sale decisions (Barbano and Lynch, 2006; Wehr and Frank, 2004).

Alkaline phosphatase (ALP) is an enzyme naturally present in milk that is inactivated at a temperature–time relationship just above that which is known to kill the most heat-resistant pathogen found in milk (*Coxiell burnetti*). Current pasteurization temperature–time relationships are set high enough to inactivate alkaline phosphatase; therefore, a positive test for this component is an indicator of inadequate heat treatment of the milk. Instrumental methods for the analysis of alkaline phosphatase in milk are available and are used by regulatory agencies as well as plants to test the effectiveness of the pasteurization process. Testing for alkaline phosphatase activity of HTST, UP, and UHT products heated to temperatures higher than 87.8°C (190.0°F) may result in false-positives due to possible reactivation of the phosphatase enzyme.

MICROBIOLOGICAL EVALUATION

The microbiological quality of the milk being received by the dairy plant is tested by the standard plate count (SPC) for total bacteria and by the preliminary incubation (PI) count for bacterial indicators of poor cleaning and sanitation procedures in the handling of the raw milk. Once the fluid milk product is pasteurized and packaged, the SPC and Coliform (Coli) counts should be run on the fresh samples; however, the tests require 48 and 24 hours, respectively, so much of the product will be in the distribution channel before the results are recorded. High SPCs in fresh samples may be an indicator of sanitation, maintenance, or refrigeration problems after the pasteurization system. The presence of Coli is an indicator of poor personal hygiene and should raise an immediate red flag with regard to supervision and training of personnel.

Samples of all packaged products should also be held for a shelf-life program that includes both microbiological and sensory evaluation of the products. The Moseley Keeping-Quality Test [Hold sample at 5–7 days at 7°C ± 1°C (45°F ± 1°F) followed by SPC] or tests providing similar information should be run on samples to help indicate the potential shelf life of the product and to help identify problem ar-

eas in the milk handling system (Wehr and Frank, 2004). The major benefit of microbiological testing will not be experienced unless regular analysis of the collected data is performed.

SENSORY EVALUATION

The first sensory evaluation of the milk should take place in the form of an aroma check at the personal access port of the raw milk tank truck by the receiver as he/she is taking the sample for receiving tests. Any off odor should be reported to a supervisor or the quality assurance manager. A sample may be taken for further evaluation but no tasting of raw milk should be performed by any personnel due to the risk of pathogenic bacteria. If after smelling the sample, the management wants a taste test to be performed; a sample should be laboratory pasteurized at 63°C (145°F) for 30 minutes prior to tasting. Often times a good sensory evaluation in the receiving room will stop a poor product from contaminating a larger pool of good product. Receiving personnel and management should be familiar with the common sensory defects and the causes related to the production of milk on the farm. A good tool for training personnel is the American Dairy Science Association (ADSA) Score Card for milk (Bodyfelt et al., in press). Although there are more powerful tools available to the sensory scientist, there is no replacement for a well-trained judge when in a process environment.

The sensory evaluation of finished product will include taste, smell, sight, and mouthfeel. Again, the ADSA Score Card is a valuable tool for the evaluation of unf flavored, fluid milk; however, other products such as chocolate milk may require the quality assurance group to develop internal company standards. A good practice is to evaluate a range of products from in-house and from competitors. Other procedures such as quantitative descriptive analysis (QDA) and consumer panels may also be valuable if developing new products but they tend to be expensive and require larger numbers of panelists. These other procedures are not able to operate effectively in the process environment where decisions need to be made in a timely fashion to keep production moving efficiently.

The primary goal of the fluid milk processor is to get a safe, high-quality product into the hands of the consumer who is the ultimate judge of the finished product. Careful performance of quality assurance programs will result in satisfied customers and a more efficient operation, both of which will result in improved returns on investment.

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10

Cultured Milk and Yogurt

T. Vasiljevic and N. P. Shah

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Fermentation Principles
Characteristics of Starter Cultures
 Mesophilic Cultures
 Thermophilic Cultures
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Cultured Dairy Products Produced by Thermophilic Lactic Starter Cultures
 Yogurt
 Bulgarian Buttermilk
 Dahi
Cultured Dairy Products Produced by Mixed Fermentation
 Kefir
 Kumys (Kumiss, Koumiss)
 Skyr
 Viili
Probiotics
 Probiotic Cultures
 Genus *Lactobacillus*
 Genus *Bifidobacterium*
 Selection of Probiotics
 Viability of Probiotics
 Improvement of the Viability of Probiotics
Health Potential of Probiotic Dairy Products
 Probiotic Effect
 Alleviation of Lactose Intolerance
 Prevention and Reduction of Diarrhea Symptoms
 Treatment and Prevention of Allergy
 Reduction of the Risk Associated with Mutagenicity and Carcinogenicity
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Inhibition of *Helicobacter pylori* and Intestinal Pathogens
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Quality Control of Cultured Dairy Products
References

INTRODUCTION

Cultured dairy products likely present the earliest form of the unintentional food preservation. The first fermented products consumed were brave attempts of a few that dared to taste “spoiled” milk. Although the realization that this bioconversion provided for prolonged stability and extended usability of food products, the sensory properties, especially mouthfeel and different flavors, were first determinants and drivers of the acceptance of these products. The origins of fermented dairy products can be traced far back to Persian times (8000 BC) in the Middle East when, as it is believed, the art of cheese making was introduced (Ross et al., 2002). On the basis of archaeological evidence, it has become apparent that civilizations that followed practiced the production of fermented dairy products such as Laban Rayeb or Laban Khad in ancient Egypt (Vasiljevic and Shah, 2007). The tradition of fermenting the milk was consequently taken with Barbarian hordes on their quests. Today, a large number of fermented dairy products exist with a relatively few having a commercial significance.

The role that fermentation played in milk preservation and sensory perception was recognized a long time ago. In the late nineteenth century, several scientists also realized that traditional cultured

products might have had additional benefits. In 1885, Escherich (1885) suggested a very important relationship between the human gastrointestinal ecology and the pathology of intestinal diseases of microbial origin. This was followed by discoveries of Tissier and Moros, who isolated two different bacteria from the feces of breast-fed infants and named them *Bacillus bifidu* and *Bacillus acidophilus*, respectively. Further follow-up studies showed that *B. bifidu* was the predominant organism in the feces of breast-fed infants approximately 3 days postpartum (Tissier, 1908) in contrast to bottle-fed neonates with the gastrointestinal microflora dominated by *B. acidophilus* (Moro, 1905). These early attempts and empirical observations of the longevity of Bulgarian peasants led Nobel Laureate Ilya Metchnikoff to propose his autointoxication theory (Metchnikoff, 2004). In his book "The Prolongation of Life," Metchnikoff postulated that microbial toxins released by pathogens in the intestine would weaken body's resistance. These detrimental processes would be prevented by the consumption of sour milk and lactic acid producing bacteria. Although a part of Metchnikoff's theory specifically underlying the role of *Lactobacillus bulgaricus*, the main organism isolated from Bulgarian sour milk, was not supported by other studies (Herter and Kendall, 1908), the diligent work of a research group at Yale University led by Prof. Leo Rettger showed that certain strains of *Lactobacillus acidophilus* were capable of colonizing human digestive tract where they exerted appreciable physiological activity (Rettger and Cheplin, 1920a,b). Since these times, the fundamental and applied research in this area has resulted in commercialization of these early findings first by introduction of Yakult, a cultured dairy product containing *Lb. casei* strain Shirota, and now a range of physiologically functional cultured dairy products such as Calpis® and Evolus®.

Although many properties of cultured products are improved through fermentation, they are still susceptible to deterioration. Traditionally, these products were made in bags made of the animal skin, which allowed for water evaporation and resulted in gradual increase in concentration of acids and total solids. The concentration of total solids accompanied in many instances with salting was applied to extend the shelf life of cultured products. This practice led to a rise of a number of different products still consumed locally. However, with the introduction of refrigeration, the interest in traditional products has declined.

VARIETIES OF CULTURED DAIRY PRODUCTS

A great number of cultured dairy products are produced worldwide; however, they may all fall under several varieties. Several attempts have been made to classify these products. Marshall (1984) used the dominating organism as a basis for classification. On the contrary, Robinson and Tamime (1990) proposed a more comprehensive list using the metabolites produced by starter cultures as a foundation. The approach, illustratively presented in Table 10.1, groups the products into three classes: lactic, yeast-lactic, and mold-lactic.

The lactic group is further subdivided into three divisions comprising of mesophilic, thermophilic, and therapeutic type of fermentation.

At the beginning of the last century, the consumption of cultured dairy products was confined mainly to specific ethnic groups, which perceived these products not only as a part of normal diet but also associated them with certain health benefits. In the last several decades, the perception of these products has changed throughout the world, especially with the introduction of different varieties such as fruit or sweet yogurt and probiotics (Tamime, 2002). The commercial trends for cultured dairy products depict the highest per capita consumptions throughout Europe and a steady increase in major markets (Table 10.2). Currently yogurt is the second most popular snack among children in the United States (Sloan, 2006).

The existing standards in many countries classify fermented dairy products mainly on the basis of the chemical composition or fat content (full, semi-skimmed, or medium, and skimmed or low). The International Dairy Federation (IDF, 1992a,b) defines fermented milks as products prepared from milk and/or milk-derived components, produced from raw materials that were at least pasteurized, by the action of specific microflora which results in a pH reduction and subsequent coagulation of casein. The IDF, additionally, recommends that (i) the starter cultures be viable, active, and abundant having concentration of at least 10^7 colony-forming units (CFU) per gram of a product throughout the shelf life; (ii) raw materials used in production are at least pasteurized, optionally homogenized, and with the optional addition of certain additives such as thickeners; (iii) final products should have a shelf life of up to 30 days stored at 4–7°C; (iv) the heat-treatment to prolong the storage stability of these products is not permitted due to the

Table 10.1. Some Commercially Available Cultured Dairy Products and Cultures Involved in the Fermentation

Fermentation Type	Product	Culture
Mesophilic	Taetmojolk	<i>Lc. lactis</i> ssp. <i>lactis</i>
	Folkjolk	<i>Lc. lactis</i> ssp. <i>lactis</i> biovar. <i>diacetylactis</i> <i>Leuc. mesenteroides</i> ssp. <i>cremoris</i>
	Ymer	<i>Lc. lactis</i> ssp. <i>cremoris</i> <i>Lc. lactis</i> ssp. <i>lactis</i> biovar. <i>diacetylactis</i>
	Cultured buttermilk and sour cream	<i>Lc. lactis</i> ssp. <i>cremoris</i> <i>Leuc. mesenteroides</i> ssp. <i>cremoris</i> <i>Lc. lactis</i> ssp. <i>lactis</i> biovar. <i>diacetylactis</i>
	Yogurt	<i>Lb. bulgaricus</i> <i>St. thermophilus</i>
Thermophilic	Acid buttermilk	<i>Lb. delbrueckii</i> ssp. <i>bulgaricus</i>
	Dahi	<i>Lb. bulgaricus</i> <i>S. thermophilus</i>
	Zabadi	<i>Lb. bulgaricus</i> <i>St. thermophilus</i>
Therapeutic	Evolus [®]	<i>Lb. helveticus</i>
	Calpis [®]	<i>Lb. helveticus</i> <i>Saccharomyces cerevisiae</i>
	Yakult	<i>Lb. casei</i> (strain Shirota)
	Gaio [®]	<i>St. thermophilus</i> , <i>Enterococcus faecium</i>
	BRA [™]	<i>Lb. reuteri</i> , <i>Lb. acidophilus</i> , <i>Bifidobacteriu infantis</i>
	Gefilu [®]	<i>Lb. rhamnosus</i> GG
	Aktifi [®]	<i>Lb. rhamnosus</i> GG
	Kefi	<i>Lc. lactis</i> , <i>Lc. lactis</i> ssp. <i>cremoris</i> , <i>Leuc. mesenteroides</i> , <i>Leuc. mesenteroides</i> ssp. <i>dextranicum</i> , <i>Lb. kefi</i> , <i>Lb. fermentum</i> , <i>Lb. brevis</i> , <i>Lb. reuteri</i> , <i>Acetobacter pasteurianus</i> , <i>Candida kefi</i> , <i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces marxianus</i>
Mixed—yeast	Kumys	<i>Lb. bulgaricus</i> <i>Lb. acidophilus</i> <i>saccharomyces lactis</i>
	Dadih	<i>Lc. lactis</i> ssp. <i>lactis</i> <i>Leuc. paramesenteroides</i> Yeast
Mixed—mould	Villi, piimi	<i>Lc. lactis</i> ssp. <i>cremoris</i> <i>Geotrichum candidum</i>

Compiled from Tamime (2002) and Vasiljevic and Shah (2007).

Table 10.2. Consumption (kg per Capita) of Cultured Dairy Products Including Yogurt in Selected Countries

Country	1980	1990	1993	1999	2000
Australia	1.8	3.5	4.8	5.8	5.7
Austria	9.8	10.4	16.4	15.1	21.4
Belgium	7.7	8.4	11.9	20.3	21.1
Canada	2.3	3.7	3.7	4.2	4.9
Denmark	26.7	21.6	20.7	29.8	26.2
Germany	10.1	14.2	24.7	25.5b	26.5
Finland	41.0	38.3	38.1	NR	40.7
France	9.3	16.4	17.3	27.4	28.5
Netherlands	27.3	32.5	29.7	NR	44.8
Norway	10.1	14.9	—	19.9	16.6
Spain	6.0	8.0	9.8	15.4	15.7
Sweden	23.5	29.1	28.6	30.2	38.0
UK	2.8	4.4	NR	NR	NR
USA	3.1	3.5	3.5	NR	2.7

Note: The data compiled from IDF (1982, 1992c, 1995, 2000, 2001).

firs requirement; and (v) the syneresis in the fina product should be avoided.

FERMENTATION PRINCIPLES

The main characteristic of these products is the use of starter cultures that bring about the chemical, sensory, and nutritional changes to a starting material, in many cases fortifie milk. The bioconversion or fermentation of traditional cultured dairy products was frequently initiated by natural, wild-type lactic acid bacteria (LAB) that originated in the raw material, processing vessels, or environment (Leroy and De Vuyst, 2004). The fermentation, in general, involves the oxidation of an organic material, mainly carbohydrates, resulting in a range of products, which include principally organic acids, alcohol, and carbon dioxide (de Vos, 1996). These metabolites provide a preservative effect by inhibiting the growth of spoilage and/or pathogenic microflor in fermented products. Additionally, other metabolites may be produced that would affect the quality of the fina fermented milk including the fl vor compounds, such as diacetyl and acetaldehyde, as well as compounds that may have positive health implications such as vitamins, antioxidants, and bioactive peptides.

LAB are typically described as Gram-positive, nonmotile, nonspore forming, catalase negative cocci, or rods. They are chemoorganotrophic, grow only on complex media, and produce lactic acid as

the major end product during sugar fermentation (Schleifer and Ludwig, 1995). The species belonging to this bacterial group that have been used in the manufacturing of cultured dairy products are mainly selected strains of *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Leuconostoc*, and *Bifidobacteriu* genera. Phylogenetically, they belong to the clostridial branch of the Gram-positive bacteria with G + C (guanine + cytosine) content less than 55 mol% in their DNA (with the exception of *Bifidobacteriu*). On the basis of their growth on glucose, members of the LAB are usually subdivided into two distinct groups. The homofermentative group consisting of *Lactococcus*, *Streptococcus*, and some lactobacilli utilizes the Embden-Meyerhof-Parnas (EMP) pathway (glycolysis) in which glucose is transformed chiefly into lactic acid. By this pathway, 1 mol of glucose is converted into 2 mol of lactate with subsequent generation of 2 mol of ATP. As opposed to homofermentors, heterofermentative bacteria produce equimolar amounts of lactate, CO₂, ethanol, or acetate from glucose-exploiting phosphoketolase pathway. The utilization of carbohydrates by this route generates only half the energy of the homofermentative group. Members of this group include *Leuconostoc* and remaining lactobacilli. The species belonging to *Enterococcus* genus are frequently encountered in traditional fermentations. However, their deliberate utilization as probiotics in cultured dairy products is still controversial, especially since some of the species have now been recognized

as opportunistic human pathogens (Franz et al., 1999).

Certainly, one of the most important traits of these cultures is the lactose utilization. Sugars, in general, can be imported into the cell by three fundamentally different transport systems: group translocation, primary transport, and secondary transport systems (de Vos and Vaughan, 1994). Bioenergetically, the most efficient system is the sugar-specific phosphoenolpyruvate-dependent phosphotransferase system (PEP-PTS), in which sugar is phosphorylated during transport. Primary transport systems are widespread and involve a sugar transport ATPase, belonging to the superfamily of ATP-binding cassette (ABC) proteins. On the contrary, sugar translocation in secondary transport systems is coupled with ions or other solutes (de Vos and Vaughan, 1994). After the sugar is imported inside the cell, it is phosphorylated and metabolized via glycolysis, also known as the EMP pathway, to pyruvate in homofermentative LAB or via 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway in heterofermentative LAB (Cocaign-Bousquet et al., 1996). In homolactics, pyruvate is further reduced mainly into lactic acid to regenerate reduced coenzymes involved in the glycolytic pathway, thus maintaining the energetic equilibrium (Fig. 10.1).

The ability of dairy starters to metabolize lactose is certainly the most important selection criterion. Lactose is imported into the cell either via group translocation transport system (PEP-PTS) or by secondary transport systems. Lactose translocated via the PEP-PTS system is phosphorylated during the transport in the cell wall and, when inside the cell, cleaved by phospho- β -galactosidase. Resulting glucose is metabolized by glycolysis and galactose is converted into tagatose via tagatose pathway (Fig. 10.1) and cleaved into trioses, entering the second stage of the glycolytic pathway. In contrast, lactose imported by secondary transport systems is first cleaved in the cytoplasm by β -galactosidase; glucose enters glycolysis, while the utilization of galactose depends on the presence of enzymes of Leloir pathway (Fig. 10.1; Cocaign-Bousquet et al., 1996). The production of ATP is achieved by substrate-level phosphorylation and reducing equivalents (NADH) at the level of glyceraldehyde 3-phosphate dehydrogenase. Reduction of pyruvate to lactate via lactate dehydrogenase (LDH) maintains the redox balance by regenerating NAD^+ . Pyruvate is the key metabolic intermediate whose conversion into lactic acid is

the main characteristic of homofermentative LAB. However, a shift toward mixed acid fermentation may occur under certain fermentation conditions, such as carbohydrate limitation, diminished rates of sugar metabolism, and aerobic conditions (Cocaign-Bousquet et al., 1996). Under these conditions, the formation of minor products from pyruvate becomes more important. This metabolism differs from a heterolactic fermentation since pyruvate is still generated via glycolysis, but further conversion involves several different enzymes such as pyruvate formate lyase (PFL) or pyruvate dehydrogenase (PDH) with formation of either formate and CO_2 or acetate and ethanol. Alternatively, very important flavor compounds such as diacetyl and acetoin may be formed. Their production is even enhanced in the presence of certain organic acids such as citrate, which is naturally present in milk. The ability of dairy LAB to metabolize citrate is very unstable trait because of the plasmid localization of genes encoding for citrate permease (Fig. 10.1; Hugenholtz, 1993). The importance of citrate metabolism is in generation of pyruvate via citrate lyase yielding acetate and oxaloacetate, which are further converted into pyruvate via oxaloacetate decarboxylase. This final step is executed without the production of reduced coenzymes. Acetoin is either excreted as an end product or is reduced to butanediol catalyzed by the enzyme acetoin reductase. Diacetyl is formed as a by-product at low pH, under aerobic conditions, from α -acetolactate by chemical decarboxylation.

Bifidobacteria as opposed to lactobacilli are nutritionally less fastidious. Carbohydrate utilization is performed exclusively by the fructose-6-phosphate shunt (Fig. 10.2).

Acetic and lactic acids are major metabolites with a theoretical molar ratio of acetic acid to lactic acid of 1.5, although the exceptions have been noted (De Vries and Stouthamer, 1968; Lauer and Kandler, 1976). The observed variations are usually due to production of other metabolites, such as formic acid and ethanol, which limits the production of lactic acid. The degradation of inulin-type fructans changes this ratio as well as the expense of lactic acid. Certain strains such as *B. animalis* DN-173 010 are incapable of metabolizing simple sugars such as glucose or fructose but not lactose, sucrose, or oligofructose, which indicates the absence of dedicated import systems required for the membrane translocation of these monosaccharides (van der Meulen et al., 2004).

Dairy LAB are fastidious in nature requiring a wide range of essential growth promoters. Although

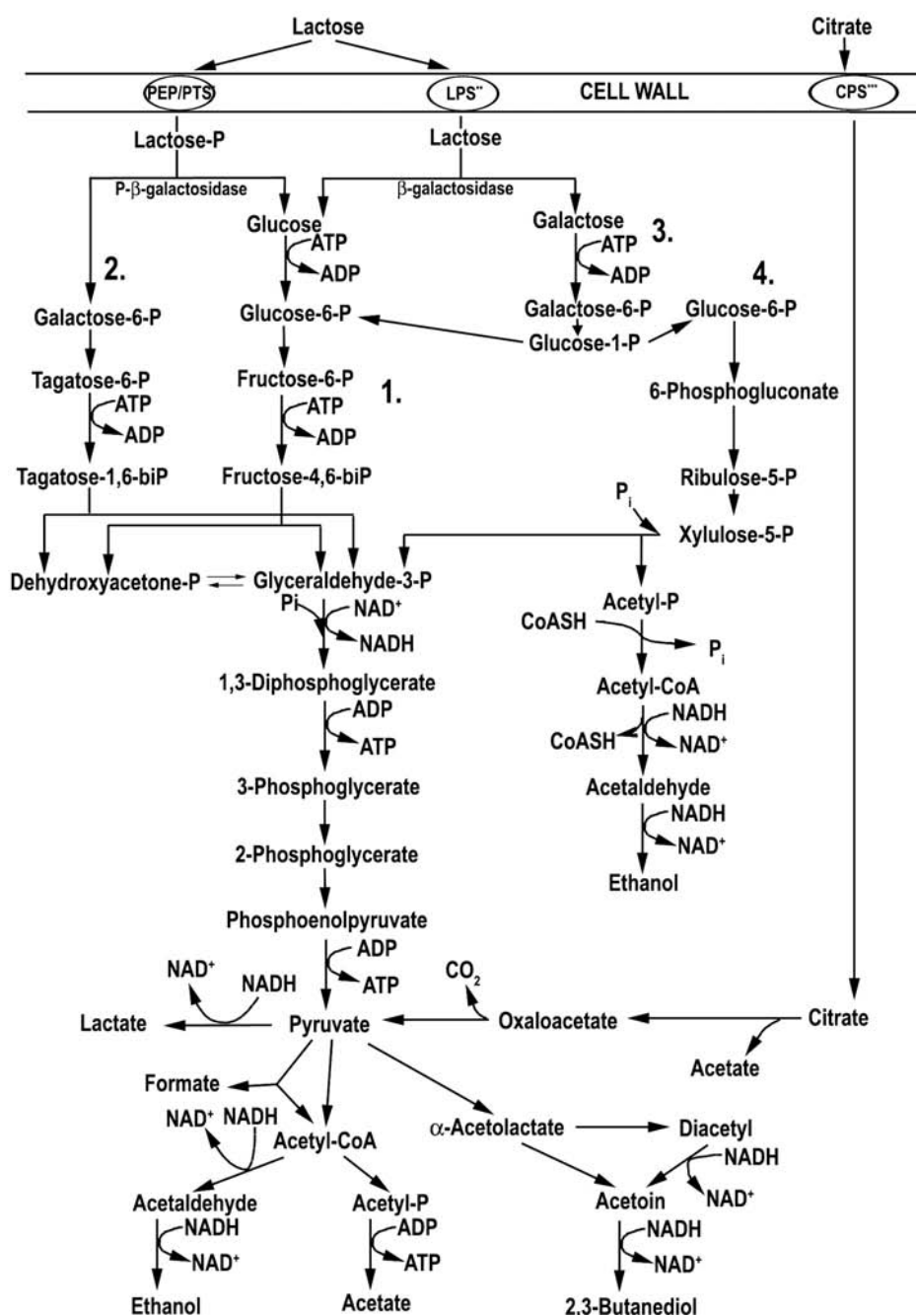


Figure 10.1. Lactose and citrate metabolism by lactic acid bacteria (LAB). (1) Embden-Meyerhof-Parnas pathway (glycolysis); (2) tagatose pathway; (3) LeLoir pathway; (4) phosphoketolase pathway. *PEP-PTS, phosphoenolpyruvate dependent-phosphatotransferase system; **LPS, lactose permease; ***CPS, citrate permease.

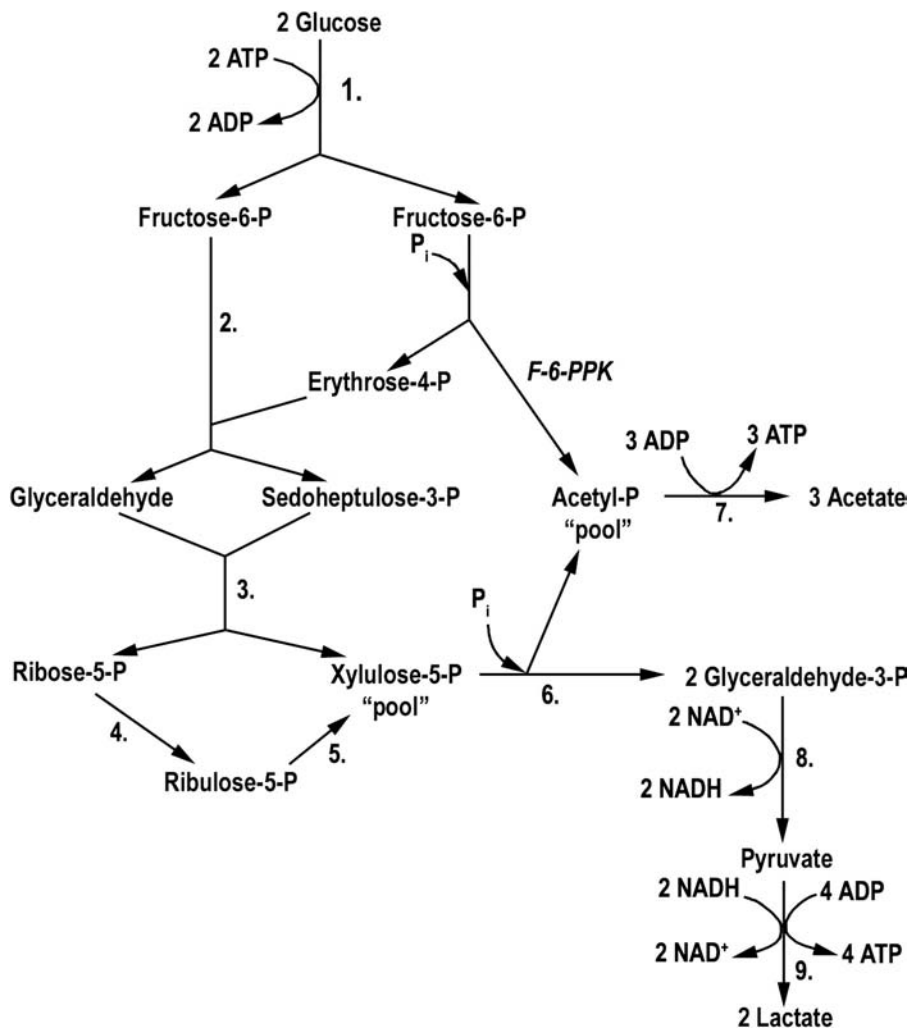


Figure 10.2. Carbohydrate metabolism in *Bifidobacterium* sp. via fructose-6-phosphate shunt. Enzymes indicated: (1) hexokinase and glucose-6-phosphate isomerase; (2) transaldolase; (3) transketolase; (4) ribose-5-phosphate isomerase; (5) ribulose-5-phosphate epimerase; (6) xylulose-5-phosphate phosphoketolase; (7) acetate kinase; (8) enzymes of the second part of glycolytic pathway; (9) lactate dehydrogenase; *F-6-PPK*, fructose-6-phosphate phosphoketolase.

considered a rich growth medium, milk contains small amounts of peptides and free amino acids to efficiently support the growth of LAB (Vasiljevic et al., 2005). In response to this limitation, LAB have developed a complex proteolytic system consisting of proteinases and peptidases, which enable them to utilize casein as an additional source of amino acids and nitrogen (Smid et al., 1991). The structural components of the proteolytic systems of LAB can be divided into three groups on the basis of their function

including proteinases (that breakdown caseins to peptides), peptidases (that degrade peptides), and transport systems (that translocate the breakdown products across the cytoplasmic membrane) (Kunji et al., 1996). The first step in casein degradation is mediated by cell wall-located proteases, which cleave caseins to oligopeptides. Further degradation to smaller peptides and amino acids that can pass through the cell membrane is performed by peptidases (Donkor et al., 2007).

The extent of proteolytic activity has important consequences on the properties of the final product. In addition to providing building blocks required for the cell development and growth, liberated peptides and amino acids also serve as precursors for the formation of important flavor compounds such as vicinal diketones, 2,3-butanedione, and 2,3-pentanedione. These aromatic compounds are formed from several amino acids including threonine and branched chain amino acids (BCAA—valine, leucine, and isoleucine; Ott et al., 2000). The proteolysis may also lead into changes of acid-induced gel. Simultaneously, the metabolic activity of starters could be affected and a range of other products may also be produced such as different exopolysaccharides that may positively or adversely affect the structural properties of products (Purwandari et al., 2007).

Dairy LAB cultures are capable of producing extracellular polysaccharides (EPS), which contribute to the texture of cultured dairy products as thickening agents (Cerning, 1995). The polysaccharides produced are either homopolysaccharides (Cerning, 1995), for example, glucans and fructans, or heteropolysaccharides (Stingele et al., 1996). The strain, culture conditions, and the medium composition affect the amount of EPS produced by certain species (Degeest and de Vuyst, 1999; Petry et al., 2000). The type of carbon source has a great influence on EPS productivity and may also affect the composition of EPS. *Lb. bulgaricus* NCFB 2772 produced three times more EPS with glucose than with fructose as a sugar source, and the type of EPS produced by this organism was influenced by the sugar source as well (Grobben et al., 1997). The amount and composition of EPS were also strongly affected by carbon/nitrogen ratio in the growth medium (Degeest and de Vuyst, 1999). Biosynthesis of EPS produced by lactococci includes the intracellular formation of EPS precursors, the sugar nucleotides, followed by the formation of a repeating unit on a lipid carrier, which is located in the cytoplasmic membrane. The last step of EPS formation involves the transport of the repeating units across the membrane to the outer layer and polymerization of several hundred to several thousand repeating units to form the final EPS (Ramos et al., 2001). While these long-chain bacterially produced polysaccharides are frequently used as thickeners, some of them, notably heteroexopolysaccharides, contain gluco- and/or fructo-oligosaccharides; thus, because of their composition, they may possess prebiotic properties and manipulate the balance of the colonic microflora. They may

also generate short-chain fatty acids upon hydrolysis in the intestinal tract which may have potentially health benefit including an anticarcinogenic, hypocholesterolemic, or immunomodulatory effects (Telang et al., 2005).

CHARACTERISTICS OF STARTER CULTURES

Dairy starter cultures are active microbial preparations added intentionally to dairy bases in order to achieve desired modifications. They have various functions in fermented dairy products which range from biopreservation, structure creation, and modification to flavor generation. These cultures may consist of single strains used alone or in combinations or undefined mixtures of strains (mixed-strain cultures). On the basis of their optimal growth temperature, they can be classified as either mesophilic (optimum temperature around 26°C) or thermophilic (optimum temperature around 42°C).

MESOPHILIC CULTURES

These cultures are represented by *Lactococcus*, *Leuconostoc*, and, to a lesser extent, *Pediococcus* genera, which are used in the manufacturing of products that undergo mesophilic lactic acid fermentation. Lactococci are Gram-positive, nonmotile, and coccus-shaped homofermentative bacteria that grow at 10°C but not at 45°C, produce L(+) lactic acid from glucose, and were thought to lack the cytochromes of the respiratory chain. However, more recently, it was shown that the presence of cytochrome D oxidase may lead to respiration, especially in the presence of heme in the medium and under certain growth conditions (Duwat et al., 2001). Several strains of this genus are well adapted to grow in milk due to acquisition of plasmid DNA encoding for the PEP-PTS system. Additional important characteristic is their ability to utilize citrate with production of diacetyl, and these strains are now classified as *Lc. lactis* biovar. *diacetylactis*. Another important culture used in fermented dairy products is *Lc. lactis* subsp. *cremoris*. *Lc. lactis* subsp. *lactis* and subsp. *cremoris* are differentiated by the ability of former to grow at 40°C in 4% NaCl and 0.1% methylene blue milk, at pH 9.2, and by formation of ammonia from arginine (Stiles and Holzapfel, 1997). The main problem with the use of these starters is in their stability due to extrachromosomal (plasmid) location of many desirable traits. The genus *Leuconostoc* is

phenotypically related to *Lactobacillus* and *Pediococcus* and share many characteristics with heterofermentative lactobacilli. Only two species of this genus are important in dairy fermentations, *Leuconostoc mesenteroides* subsp. *cremoris*, and *Leuconostoc lactis*. These cultures are facultative anaerobes with complex growth requirements (Stiles and Holzapfel, 1997). They prefer mild acidic conditions with the temperature optimum between 18 and 25°C. Lactose is metabolized via phosphoketolase pathway and the composition of end products depends on the presence of oxygen in the medium: microaerophilic conditions yield lactate, ethanol, and CO₂, while acetate and twice the amount of ATP replace ethanol under aerobic conditions. Their deployment in cultured dairy products is based on their ability to convert citrate to diacetyl, carbon dioxide, and acetoin.

THERMOPHILIC CULTURES

The most important cultures used in the production of cultured dairy products which undergo thermophilic lactic fermentation belong to *Lactobacillus* and *Streptococcus* genera, specifically *Lactobacillus* (*Lb.*) *delbrueckii* subsp. *bulgaricus* (commonly referred to as *Lb. bulgaricus*) and *Streptococcus* (*St.*) *thermophilus*. *Lb. bulgaricus* is a Gram-positive, non-spore forming, nonmotile rod, 0.5–0.8-μm thick and 2–9-μm long. It grows well at 45°C, but is unable to grow at or below 15°C. Though anaerobic, it is also capable of tolerating and even growing in aerobic environments (Bury et al., 1998). As an obligate homofermenter, *Lb. bulgaricus* metabolizes lactose to lactic acid via the EMP pathway but is unable to utilize pentoses or gluconate (Hammes and Vogel, 1995). The galactose moiety of lactose is not utilized and is excreted outside the cell by the secondary lactose–galactose antiport system. The optimal pH for the growth of *Lb. bulgaricus* in the lactose-modified MRS broth is 5.8 (Venkatesh, 1998). Product inhibition was evident when the concentration of lactic acid exceeded 60 g/L, while lactose was inhibitory at concentrations over 80 g/L. *Lb. bulgaricus* is unable to synthesize many of the essential compounds it requires, and depends on the availability of amino acids, vitamins, purines, pyrimidines, and other factors in a culture medium for growth. The biosynthetic ability of lactobacilli is so limited that even humans have simpler vitamin requirements. Therefore, *Lb. bulgaricus* grows relatively slowly in whey or whey permeate, but grows well in milk (Vasiljevic and Jelen, 2001). However, the

amount of free amino acids and peptides in milk is very low, resulting in the dependence of *Lb. bulgaricus* on a proteolytic system allowing the degradation of milk proteins for growth (Juillard et al., 1995).

Streptococci were early recognized and associated with a large number of human and animal diseases. The only species within this genus adapted to dairy environment is *St. thermophilus*. It gives negative response to a Lancefield group antigen and grows at 45°C and up to 50°C but not at 15°C, and is relatively heat resistant (Stiles and Holzapfel, 1997). Its primary function in dairy fermentations is the rapid conversion of lactose to lactic acid with simultaneous production of flavor compounds. *St. thermophilus* has fairly limited ability to ferment a number of sugars. In contrast to other LAB, glucose is a non-PTS carbon source for *St. thermophilus* and is a poor substrate for growth (Poolman, 1993). Sucrose is one of the few sugars imported by a PTS and provides for rapid growth, although maximal growth rates are achieved in media containing lactose. *St. thermophilus* is highly adapted to growth on lactose due to the presence of the lactose-dedicated permease LacS (Foucaud and Poolman, 1992). Despite efficient transport and intracellular hydrolysis, galactose moiety is not utilized by most of the strains and is excreted into the medium in amounts stoichiometric with the uptake of lactose.

St. thermophilus and *Lb. bulgaricus* are used together in production of yogurt and cultured dairy products with the yogurt base due to their associative and symbiotic growth. The symbiosis results in a greater growth rate and faster lactic acid production in comparison to those of the individual cultures (Tamime and Robinson, 1999). Upon inoculation, *Lb. bulgaricus* would initiate growth first and start releasing short-chain peptides and free amino acids and thus promoting the growth of *St. thermophilus*. Because of efficient lactose uptake, *St. thermophilus* would continue growing more rapidly producing formate and modifying the environment by release of carbon dioxide. These two compounds would consequently provide for the enhanced growth of *Lb. bulgaricus*, and the bulk of acid toward the end of fermentation is produced by this organism. High concentration of lactic acid favors *Lb. bulgaricus* due to its acidophilic nature, but it also adversely affects *St. thermophilus*, whose growth would eventually cease. The overproduction of acids during storage may lead to a defect commonly referred to as post-acidification (Donkor et al., 2006).

THE MANUFACTURE OF YOGURT AND OTHER CULTURED DAIRY PRODUCTS

At present, a number of different cultured dairy products exists on the market. In addition to classification based on the type of starter cultures involved in the processing, another way to group these products is based on the state of water and includes gel/liquid, concentrated/strained, frozen, or dried products. The quality of cultured products varies with the composition and microbial quality of the raw materials, addition of ingredient, unit operations involved, and handling of the coagulum after fermentation. Despite the variety of products, the steps involved in the manufacturing are fairly similar and could be summarized in the following: standardization of the milk base, homogenization, heat treatment, starter culture addition, and cooling.

The milk base is standardized to achieve required legal standards in regard to protein and fat content. The fat content can be very low as in case of low-fat products or relatively high as in cultured cream although the latter is rather used for dips and toppings than for the direct consumption. Furthermore, the concentration of total solids can be increased by evaporation, by direct addition of skim milk solids such as skim milk powder and skim milk concentrate, or by membrane processing such as reverse osmosis (Sodini et al., 2004). With increase in total solids concentration, perceived thickness and firmness of products would also increase. Supplementation with whey proteins may lead to textural defects, most noticeably extent of syneresis due to weakening of the acid-induced gel (Lucey, 2002). In many commercial cultured dairy products, other compounds such as stabilizers and sweeteners are added to impart desired textural or flavor characteristics.

The milk base used in production of cultured products can be considered as true solution of lactose and salts, colloidal suspension of proteins, and oil in water (O/W) emulsion of milk fat. Therefore, homogenization is applied in order to stabilize such a complex system and to prevent thermodynamically driven phase separation. In this process, a positive displacement piston pump is used to draw a mix through a check valve into the pump cylinder. On return of the piston, the mixture is forced through the adjustable annular gap of a discharge valve and impinges on an impact ring. The impingement and impact are the main factors in the size reduction, although the hydrodynamic cavitation may also play a role. The main effects of homogenization are

reflect in the fat globule size reduction below 2 μm which slows down the rate of coalescence and separation; viscosity increase due to the positioning of proteins on the surface of fat globules; improved whiteness of the product and diminished syneresis due to increased hydrophilicity and water holding capacity of milk proteins (Lucey, 2004). Native fat globules decrease firmness of acid gels by interfering with the network formation (Aguilera and Kessler, 1988). The placement of milk proteins on the surface of fat globule membrane provides for greater stability and crosslinks such stabilized globules with the gel matrix (van Vliet and Dentener-Kikkert, 1982). Typically, the homogenizer is placed upstream (before heat treatment) and the operating pressure ranges between 15 and 20 MPa and temperature 60–90°C. The operating conditions depend on the type of the products, that is, buttermilk is homogenized at low pressure (5–10 MPa) and 20°C (Tamime, 2002).

Traditionally, boiling of milk base in an open vat was practiced mainly to increase the solid content, although other effects were also accomplished. Current heat processing is aimed at achieving several important goals including the creation of a favorable environment for starter culture by eliminating undesirable microflora and releasing stimulatory factors and induction of various physicochemical changes that will impact on the properties of the final product (Lucey, 2004). The extent of heat treatment depends on the required effects and time. The elimination of the vegetative microorganisms reduces competition and insures a good growth medium for starter cultures. The release of certain stimulatory or inhibitory growth factors appears to be related to liberation of denatured nitrogenous compounds such as cysteine, glutathione, or thioglycolate. The expulsion of oxygen due to heating also has a stimulatory effect (Tamime and Robinson, 1999). The extent of heat treatment is usually greater than it is necessary to achieve desired preservation effect. During yogurt manufacturing, the heating of yogurt base at 90–95°C for 5–10 minutes results in increased firmness of the acid gel and reduced syneresis. These physical changes can be related to the extent of denaturation of the whey proteins (Lucey et al., 1999). Several studies have shown that during this extensive heating, aggregates are formed between κ -casein and denatured whey proteins. The composition and concentration of the whey protein/ κ -casein complexes are dependent on the heating conditions and soluble and micelle-bound forms could be isolated (Guyomarc'h et al., 2003). Initially the aggregates of β -lactoglobulin and α -lactalbumin are formed approximately in the ratio 3:1

and subsequently they would interact with κ -casein. Higher whey protein/casein ratio in the milk gives larger soluble aggregates. This complex aggregation is associated with the higher gelation pH, stronger gel strength, and fine microstructure. The heat denatured whey proteins initiate early gelation at high pH values which is followed by the gelation dominated by casein–casein interactions as acidification proceeds (Lucey et al., 1998a).

The heat denaturation of whey proteins also results in a modified kinetics of acid-induced casein gelation shifting the pH at the gelation point from 5.1 in raw milk to 5.5 in heat-treated milk (Horne, 1999). During acidification various physicochemical changes of caseins take place. Caseins are the major milk proteins composed of four types, namely, α_{s1} , α_{s2} , β , and κ -casein, which exist in the milk in an aggregated form referred to as casein micelle. These aggregates also contain large amount of calcium and phosphorus in the form of colloidal calcium phosphate (CCP), which plays an important role in maintaining the integrity of the casein micelle in addition to soluble Ca^{2+} and hydrogen and hydrophobic bonding (Lucey and Singh, 1997). Lowering of pH results in solubilization of CCP and leaching of caseins into the milk serum phase. The aggregation occurs around pH 4.6, which is the isoelectric point of caseins (Lucey and Singh, 1997). The building blocks of the gel can be represented in the form of fractal clusters and described using the fractal aggregation theory. This theory states that a fractal-scaling regime would occur only over small length scales, in the order of the aggregating clusters. At higher scales, this microstructural organization appears homogeneous. The fractal spherical particles can move by Brownian motion and may aggregate with other particles upon collision (Lucey and Singh, 1997).

In addition to parameters described above, the textural properties of cultured dairy products are affected by the inoculum size and incubation temperatures, which directly affect the rate of acidification. The viscoelastic properties of these acid gels are most frequently assessed using rheological measurements, specifically small amplitude oscillatory shear. In situ measurement of gelation kinetics during yogurt fermentation revealed that storage modulus (G'), which describes the elastic properties of a gel, increased with lowering of the incubation temperature (Kristo et al., 2003; Lee and Lucey, 2004). Conversely, the maximum values of loss tangent were attained with an increase in incubation temperature (Lee and Lucey, 2004). Higher incubation temperatures resulted in acid gels with greater

whey separation. Different microstructural properties of acidified milk were observed when different acidifiers were used, likely because of differences in the pH profile and the rate of acidification (Lucey et al., 1998b). The rate of pH change, which is dependent on the inoculum size and optimum growth temperature, affects the integrity of the casein micelle causing changes in internal micellar organization, which subsequently govern the viscoelastic properties of acid-induced gels. The texture of cultured dairy product may also be modulated by addition of exopolysaccharide (EPS) producing cultures. The EPS produced by starter cultures improve sensory characteristics such as mouthfeel, shininess, clean cut, ropiness, and creaminess (Folkenberg et al., 2005). The ultimate characteristics are, however, strongly dependent on the structural properties of the EPS, such as type-capsular or ropy (Bouzar et al., 1997; Hess et al., 1997), the degree of ropiness (Hassan et al., 1996), sugar composition (Petry et al., 2003), and degree of branching (Rinaudo, 2004). The role of the capsular and ropy EPS on the texture of yogurt has been extensively studied for their distinctly different behaviors in relation to the interaction with milk proteins during yogurt manufacturing. They differed in their localization within the protein network (Folkenberg et al., 2005; Hassan et al., 2003) and their effect on the viscosity and consistency (Hassan et al., 2003). The temperature of fermentation may affect the viscosity directly (Lee & Lucey, 2004) or indirectly via bacterial EPS production (Ruas-Madiedo et al., 2002). The EPS production also appears to be temperature dependent (Haque et al., 2001), although the viscosity of yogurt was not always directly proportional to the EPS concentration (Ruas-Madiedo et al., 2002; De Vuyst et al., 2003). For example, the relatively low EPS concentration produced by *L. lactis* ssp. *cremoris* B40 resulted in a viscosity similar to that given by the strain yielding the highest EPS concentration (Ruas-Madiedo et al., 2002). Also, yogurt with the highest EPS content had the lowest viscosity (Bouzar et al., 1997). Furthermore, the application of EPS-producing yogurt cultures may decrease the extent of syneresis, which is likely to be due to enhanced water-binding ability (Hess et al., 1997; Moriera et al., 2000).

The main goal of fermentation is the provision of product stability due to acidification activity of the starter cultures. When the ultimate pH is reached (≈ 4.6), the product is quickly cooled to diminish the metabolic activity of the culture, otherwise certain defects may occur. For example, *Lb. bulgaricus* would predominate in the mixed yogurt culture

and continue producing lactic acid. This may lead to excessive acidification and defect called post-acidification and in certain instances, titrable acidity of 2% may be achieved. The production of acetaldehyde may also be enhanced resulting in overly “green” fl vor.

CULTURED DAIRY PRODUCTS PRODUCED BY MESOPHILIC LACTIC STARTER CULTURES

These products, illustratively presented in Table 10.1, are produced by metabolic activity of lactic starters, whose growth optimum is between 20 and 30°C. The main representatives of this group are cultured buttermilk, Scandinavian sour milk products, and sour cream.

CULTURED BUTTERMILK

It is a lightly salted fermented product prepared usually from low-fat milk, although fat-free and whole milk varieties exist. This product differs from normal buttermilk, which is an aqueous phase collected after churning of cream during butter making. It is rich in phospholipids as a consequence of rupture of fat globule membrane rupture and is considered an excellent emulsifier. The relation between these two products is in the use of the mixed mesophilic starter culture (Table 10.1), which produces diacetyl and acetic acid in addition to lactic acid. The flow diagram of cultured buttermilk production is depicted in Figure 10.3.

The milk base is first standardized to a desired level of nonfat solids (10–12%). *L. lactis* subsp. *lactis* utilizes lactose-producing lactic acid, while other two cultures are mainly responsible for the fl vor generation and the presence of sufficient amounts of citric acid to produce diacetyl (Fig. 10.1). Therefore, the milk base is usually enriched by addition of sodium citrate (0.1–0.15%). Acidic conditions are favored by fl vor producers and diacetyl production starts when pH reaches 5. The standardized milk base is heat treated at 85–88°C for 30 minutes, cooled to ≈60°C and homogenized at 18–20 MPa. The pasteurized and homogenized mix is consequently cooled to 22°C and inoculated with mixed starter culture for approximately 14–16 hours. The fermentation is terminated when the acidity reaches 0.85–0.90%. The coagulum is thoroughly stirred and homogenized at low pressure (5–10 MPa) resulting in a viscous and pourable product. Cultured buttermilk is a delicate product,

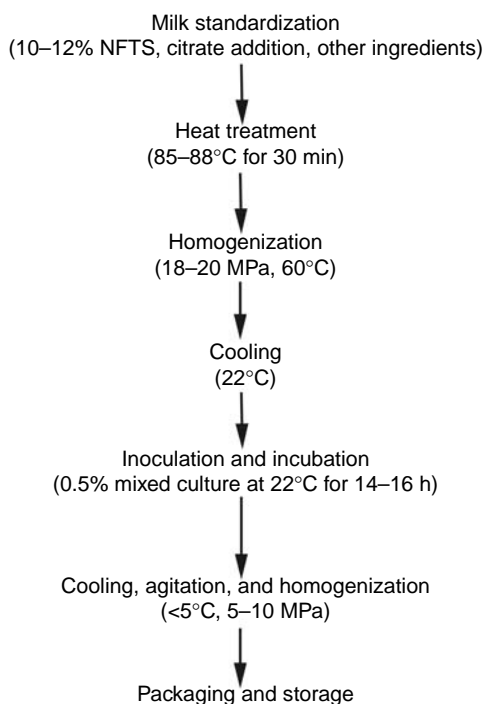


Figure 10.3. The flow diagram for the manufacturing of cultured buttermilk.

which quality may be easily affected if proper handling and manufacturing procedures are not followed. Quality defects may result from improper culture usage as well as poor manufacturing practice. Culture-related defects can be fl vor defects and may indirectly lead to body defects. These defects can be prevented by insuring proper culture activity since the main fl vor compound, diacetyl, is produced only after sufficient acid production (0.8–0.85%, pH 5) and incubation at 22°C. Supplementation with citrate appears important, and diacetyl degradation should be prevented after fermentation by proper cooling and gentle stirring. Post-acidification may also lead into diacetyl degradation, overly acidic fl vor, and extensive syneresis.

SOUR CREAM OR CULTURED CREAM

It is a fermented fat-rich product with a pleasant acidic taste and fl vor similar to that of the cultured buttermilk. The starter culture used in its production is similar to that for cultured buttermilk. The main

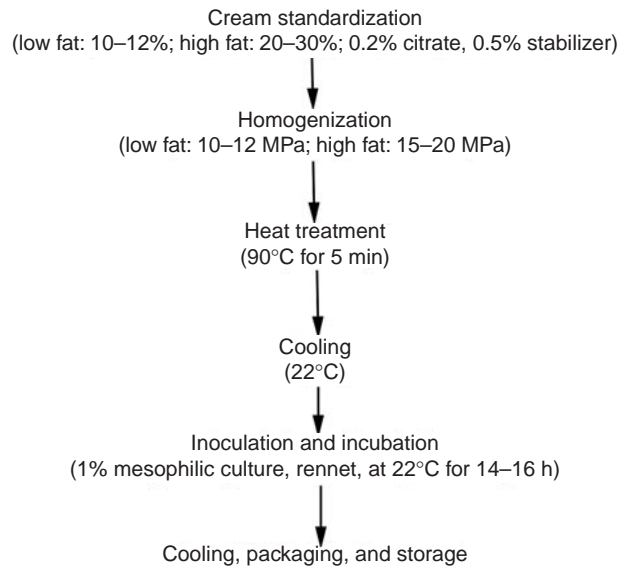


Figure 10.4. The flow diagram of sour cream manufacturing.

characteristics of this product are thick and heavy body due to high-fat and solids contents with a clean acidic and slight diacetyl flavor. The flow diagram of sour cream production is schematically presented in Figure 10.4.

The cream is first standardized to a desired fat content, followed by heating to approximately 70°C, at which temperature it is homogenized usually twice to produce a smooth product of desired viscosity. The pasteurization is the next step in the manufacturing upon which the product is chilled and inoculated with the defined starter culture. The incubation is performed at 22°C for 14–16 hours. The fermentation is terminated when the ultimate pH is reached (usually 0.7–0.9% lactic acid) by cooling the product down to 4°C. Sour cream is a fat-rich product, which makes manufacturing of low-fat varieties extremely difficult. To compensate for fat reduction, various thickening agents are used.

SCANDINAVIAN CULTURED DAIRY PRODUCTS

These include traditional buttermilk products produced byropy starter cultures with frequent inclusion of herbs. In general, the manufacturing of these products is similar to production of buttermilk with the use of mixed culture consisting mainly of *Lactococcus* spp. with inclusion of *Leuconostoc mesen-*

teroides subsp. *cremoris* for flavor and aroma production. These products are known under their traditional names as tättmjölk, tättmjölk, film ölk, tättfil långjolk, and filunk (Tamime and Marshall, 1997).

Ymer is a cultured dairy product of Denmark with a high protein (5–6%) and fat (3.5%) content. The starter culture used for its production is similar to buttermilk culture and consists of *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. mesenteroides* subsp. *cremoris*. Currently, ymer is commercially produced using ultrafiltration in order to increase milk solids prior to fermentation (Tamime and Marshall, 1997). Before fermentation, standardized and concentrated milk is homogenized (23 MPa) and pasteurized (90°C for 5 minutes). The base is then cooled, inoculated with the starter, and incubation takes place at 20–22°C for 20 hours. The final product has a pleasant acidic and diacetyl flavor (Tamime and Marshall, 1997).

CULTURED DAIRY PRODUCTS PRODUCED BY THERMOPHILIC LACTIC STARTER CULTURES

This group of products is likely commercially the most important and involves the fermentative ability of the starter cultures, which grow in thermophilic temperature range, frequently above 37°C.

YOGURT

It is the most popular contemporary cultured dairy product. It owes its popularity to a great variety of products manufactured from the yogurt base, which can be divided on the basis of their nutritional characteristics (full, low, or nonfat, low calorie) or textural characteristics (set, blended, or stirred, fruit on bottom, liquid). It has been widely accepted that the origin of yogurt is the Balkan Peninsula and the Middle East. Initially traditional yogurt, which is plain and unsweetened with a sharp acidic flavor, was poorly received by other communities outside these areas. However, its popularity had increased substantially with the introduction of fruit and sweetened yogurt in 1950s. This has been later supported with adequate marketing campaign and inclusion of probiotics which further improved health-related perception of yogurt (Tamime and Robinson, 1999). The definition and specification of yogurt can be found, in particular, legal standards, that is, yogurt in the United States is defined as a food produced by culturing dairy ingredients specifically with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lb. bulgaricus* and *St. thermophilus* (Code of Federal Regulations, 2006), while the Codex Alimentarius (2003) defines it as a milk product obtained by fermentation by the action of symbiotic culture, *Lb. bulgaricus* and *St. thermophilus*. Alternatively, yogurt can be produced with *St. thermophilus* in conjugation with any *Lactobacillus* species.

Yogurt manufacture includes several steps including standardization of the yogurt base, homogenization, heat treatment, cooling to incubation temperature, inoculation with yogurt cultures, incubation, cooling, and packaging. The last three steps depend on the type of yogurt produced and these differences are depicted in Figure 10.5.

The role each step plays in defining the quality of the final product is described in greater detail in the previous section. Briefly, high-quality milk is used as a base, which is standardized for fat content, depending on the legal requirement, and total solids non-fat (TSNF). While in many instances, standardization is performed to protect consumers, manufacturers may also decide to use a different level of TSNF to achieve desired physical properties or flavor. For example, consistency of yogurt has a great importance and is directly related to the level of total solids (TS) in the yogurt. However, the TS level is rarely higher than 16% since above this TS content the consistency appears

to be little affected (Tamime and Robinson, 1999). Standardization is most frequently achieved not only by direct addition of butterfat and milk solids not fat but it can be achieved also by concentration (evaporation and ultrafiltration). After standardization, the yogurt base is heated to 60–65°C and subjected to double-stage homogenization (10–17/3.4 MPa). International standards require that yogurt be produced from pasteurized milk. As described in the previous section, the heat-treatment applied in the yogurt manufacture is well above the required level to achieve a desired pasteurization effect and performed at 80–85°C for 30 minutes or 90–98°C for up to 7 minutes (Lucey, 2004). The pasteurized yogurt base is then cooled down to 40–45°C and inoculated with 2–3% the mixed yogurt culture, most frequently consisting of strains of *Lb. bulgaricus* and *St. thermophilus*. From this point, the processing flow will depend on the type of yogurt produced. The production of set-type yogurt involves dispensing the inoculated yogurt base into retail containers, followed by incubation at 40–45°C for 2–4 hours until desired titratable acidity (0.9%) is reached. In contrast, the stirred or Swiss-type yogurt is produced by in-vat fermentation at 40–45°C, followed by agitation and packaging of stirred coagulum into retail containers. After fermentation, the yogurt is cooled down and stored at 2–5°C mainly to slow down the metabolic activity of the culture. In the case of set-type yogurt, cooling is accomplished by placing containers directly to a cold store. Alternatively, they can be blast chilled first. The stirred yogurt can be cooled during agitation step in the vat. Although the coagulum is broken, the network is reestablished fairly quickly (Lucey, 2004). The fruit and other ingredients can be added after initial cooling, followed by filtering and container filling. Such a packaged product is blast chilled and stored at cold temperatures.

Because of the low pH, yogurt is less prone to quality deteriorations caused by contaminants of microbial origin. Severe heat treatment creates favorable conditions for the culture growth, which in addition to lactic acid produces a range of antimicrobial compounds that preserve the product. However, yogurt still may suffer from common defects such as high acidity due to post-acidification and distinct acetaldehyde flavor. These defects mainly develop due to improper manufacturing practice and poor storage conditions, which allow for the continuous proliferation of lactobacilli and excessive production of lactic acid. This may be prevented by inoculating the yogurt base with the yogurt culture in a ratio of 1:1

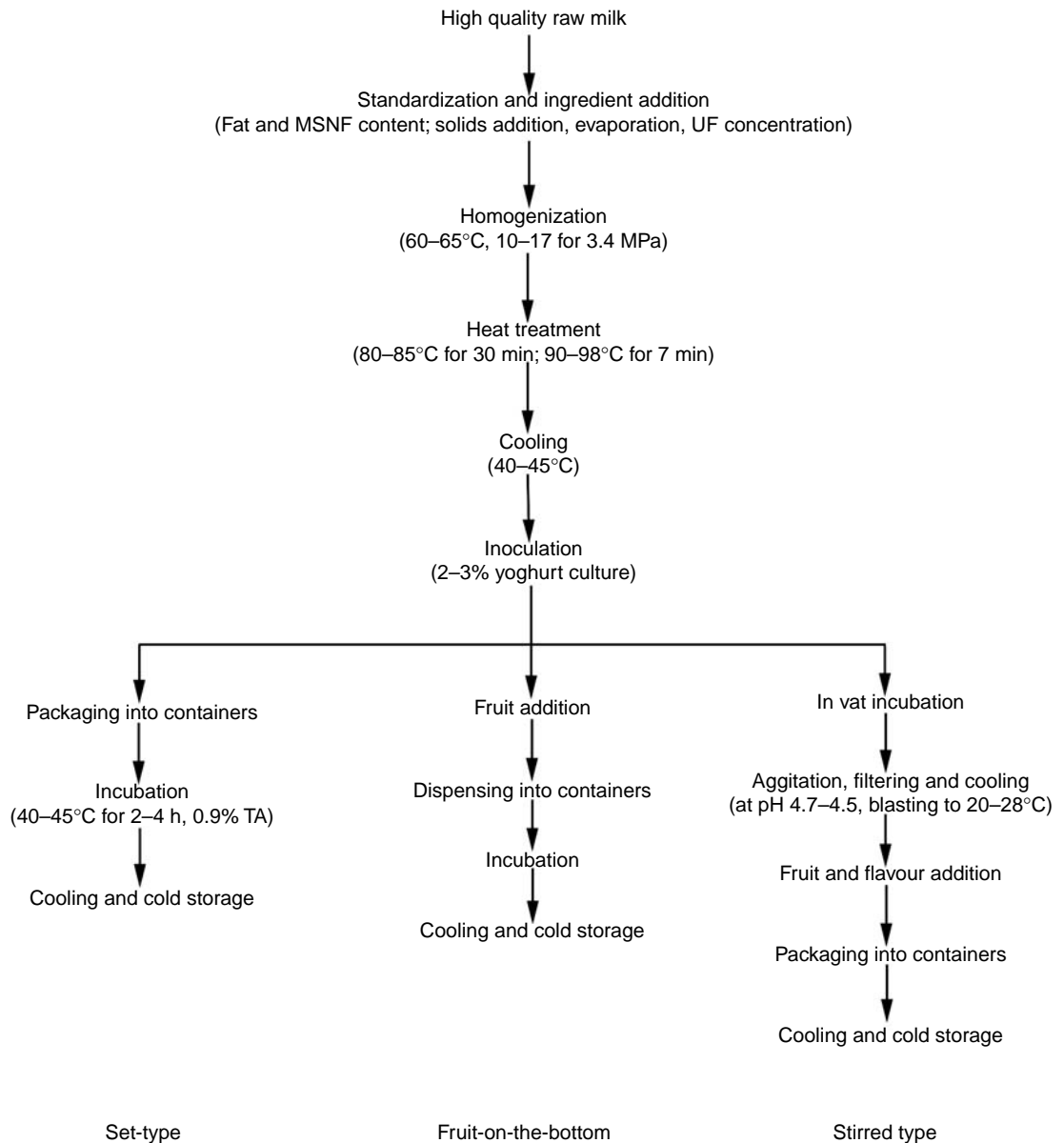


Figure 10.5. The flow diagram of manufacture of various types of yogurt. (Adapted from Lucey, 2004; Tamime and Robinson, 1999.)

between *St. thermophilus* and *Lb. bulgaricus* as well as by the rapid cooling after incubation to diminish the metabolic activity of lactobacilli. The excessive metabolic activity especially proteolytic activity of the yogurt culture may also result in bitterness. This may also be caused by the presence of spore-

formers such as *Bacillus subtilis* or *B. cereus*, which survive the heat treatment. Addition of contaminated fruits may also cause the cross-contamination of yogurt by yeast and molds. Yogurt should be smooth, free from lumps, and spoonable (Tamime and Robinson, 1999). Some common textural defects include

weak body, syneresis, and lumpiness. The weak body is caused by low solid and stabilizer concentration in the yogurt base, insufficient heat treatment to allow for whey protein denaturation, high incubation temperatures, and improper post incubation handling causing irreversible structural breakdown. Syneresis is whey expulsion due to shrinkage of the coagulum. High incubation temperature, unbalanced whey protein to casein ratio, and improper handling of the product during storage and distribution are some of the reasons that result in a product with excessive syneresis.

BULGARIAN BUTTERMILK

It is a high acid product, mainly produced in Bulgaria. It is made by fermenting pasteurized (85°C for 30 minutes) milk with *Lb. bulgaricus* alone at 40–42°C for 12–16 hours. The fermentation is stopped when about 1.4% titratable acidity is achieved. The flavor of the final product resembles that of yogurt and is dominated by acetaldehyde.

DAHI

Dahi is a cultured dairy product of major importance in the Indian subcontinent. It is still made in every household in villages using traditional method (Prapatti and Nair, 2003). The product is typically made by fermentation of the cow or water buffalo milk with thermophilic starter culture. It has a texture and flavor similar to those of yogurt. The final quality of the product tends to vary because of the practice of the culture back-slopping, that is, use of a part of the previous day product. The manufacturing of *dahi* is fairly simple and consists of the base preparation followed by brief boiling, cooling to room temperature, and inoculation with 0.5–1.0% culture. The incubation is carried out at room temperature for 12–16 hours.

CULTURED DAIRY PRODUCTS PRODUCED BY MIXED FERMENTATION

This group of cultured dairy products comprises of products fermented by mixed lactic starter and lactose and/or nonlactose fermenting yeast and mold. These products are rather contained to specific areas, consumed locally and, in some instances, there is little commercial importance. The origin of these products is frequently located in the areas of the former

USSR. Considering the commercial significance kefir is likely the most important.

KEFIR

It is a refreshing drink originating from the Caucasian mountains of the former USSR. It is also manufactured under a variety of names including kephir, kiaphur, kefer, knapon, kepi, and kippi with artisanal production of kefir occurring in countries as widespread as Argentina, Taiwan, Portugal, Turkey, and France (Farnworth, 2005). Traditionally, kefir is produced by addition of kefir grains to cow's milk. The kefir grain is composed of a diverse spectrum of species and genera including LAB (*Lactobacillus*, *Lactococcus*, *Leuconostoc*), yeasts (*Kluyveromyces*, *Candida*, *Saccharomyces*, and *Pichia*), and sometimes acetic acid bacteria (acetobacter) in a symbiotic association. This microflora is embedded in a heteropolysaccharide matrix (kefiran composed of equal amounts of glucose and galactose (Rea et al., 1996). The presence of each of these groups results in noticeable effect on properties of the final product. The size of the inoculum governs the pH, viscosity, and microbiological profile of the final product. The continuous production was achieved by either back-slopping, adding fresh milk to small quantities of kefir, or sieving off of kefir grains that would subsequently be reused in the production of new batches (IDF, 1984). Fermentation with the inoculum proceeds for approximately 24 hours and involves the rapid growth of homofermentative streptococci causing a pH decline favored by lactobacilli, which would outgrow streptococci. Lower fermentation temperature (21–23°C) favors yeasts and aroma-producing heterofermentative LAB. The flow diagram of kefir production involving the use of kefir grains is schematically shown in Figure 10.6.

Kefir grain is a key factor in the production of kefir with a dynamic and complex microflora. Therefore, the final product has frequently a different microbiological profile from the starting grains and prevents inoculation a new batch of milk and commercial application of kefir grains. Attempts have been made to resolve this situation by use of pure cultures. Beshkova et al. (2002) produced a starter consisting of two bacterial species (*Lb. helveticus* and *L. lactis* subsp. *lactis*) and yeast (*S. cerevisiae*) isolated from kefir grains in combination with yogurt-mixed culture (*Lb. bulgaricus* and *St. thermophilus*). Similarly, kefir is commercially produced in the United

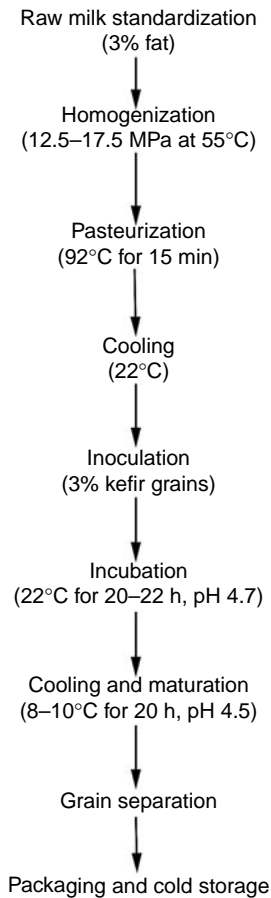


Figure 10.6. The schematic representation of traditional kefir manufacture.

States using a mixture of defined microorganisms (Farnworth, 2005).

KUMYS (KUMISS, KOUMISS)

It is another fermented milk product produced by mixed yeast—lactic fermentation originally of mare's milk. Similar to kefi, kumys has a long tradition dating some 25 centuries back when the Scythian tribes enjoyed benefit of this refreshing drink (Koroleva, 1991). Traditionally, fresh mare milk was filled into leather sacks and agitated with a wooden paddle. The mixed culture involved in the fermentation consisted of several types of yeasts, such as *Saccharomyces lactis* and *Sac. cartilaginosus*, and thermophilic strains of *L. delbrueckii* subsp. *bulgaricus*

and mesophilic strains of *L. caucasicum* (Koroleva, 1991). Because of the presence of yeast, kumys may contain substantial amounts of alcohol (up to 2.5%). Carbon dioxide is also produced by the yeast. Traditionally, the milk is not heat-treated; hence a high level of starter culture (30%) is used. The incubation is carried out at 26–28°C and depending on the fermentation time the product may contain 0.6% lactic acid and 0.2% alcohol, 0.8% lactic acid and 1.5% alcohol, or 1.0% lactic acid and 2.5% alcohol.

SKYR

It is a concentrated cultured dairy product produced from skim milk and very popular in Iceland. The product is traditionally produced by cheese-cloth method to separate the whey. The commercial method uses the nozzle separator for the solid concentration. The culture involved in fermentation consists of *Lb. bulgaricus*, *Lb. Helveticus*, and a lactose fermenting yeast. The fermentation is conducted in two stages to facilitate the culture growth in succession. The first stage is performed at a higher temperature, usually 40°C, which favors the growth of thermophilic lactic starter culture for 4–5 hours, which is followed by cooling and fermentation at 18°C for 18 hours to promote the growth of the yeast (Tamime and Marshall, 1997).

VIIILI

It is a very popular cultured dairy product in Finland. The fermentation is primarily achieved by the culture consisting of *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* ssp. *cremoris* and lactose fermenting mold *Geotrichum candidum*. The cream layer is usually covered with the mold and the product is eaten with a spoon. Pasteurized milk is fermented at approximately 20°C until a final acidity of 0.9% is reached (Tamime and Marshall, 1997).

PROBIOTICS

The early work of Tissier, Moro, Metchnikoff, and Rettger had paved the road for other scientists who with the aid of contemporary analytical techniques started elaborating further on beneficial properties of several microbial species. The efforts resulted in the introduction of the probiotic concept, which are currently defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002). The word

“probiotics” was initially used as an antonym of the word “antibiotic.” It is derived from Greek words $\pi\rho$ and $\beta\iota\omicron\tau\omicron\varsigma$ (pro biotos) and translated as “for life” (Hamilton-Miller et al., 2003). The origin of the first use can be traced back to Kollath (1953), who used it to describe the restoration of the health of malnourished patients by different organic and inorganic supplements. A year later, Vergin (1954) proposed that the microbial imbalance in the body caused by antibiotic treatment could have been restored by a probiotic-rich diet; a suggestion cited by many as the first reference to probiotics as they are defined nowadays. Late 1980s and 1990s saw a surge of different definitions of probiotics. Most frequently cited definition is that of Fuller’s, who define them as “a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1992). Although many authors suggested that probiotics should include live microorganisms, Salminen et al. (1999) offered their view and provided evidence for incorporating nonviable bacteria in the definition.

PROBIOTIC CULTURES

Probiotic cultures have been used extensively for the development of a range of products with various functional properties. Approximately 70 probiotic-containing products are marketed in the world (Shah, 2004). Dairy systems have been traditionally used as delivery vehicles for probiotics with a number of other carriers for probiotic examined including mayonnaise (Khalil and Mansour, 1998), edible spreads (Charteris et al., 2002), and meat (Arihara et al., 1998). Commercial cultures used in these applications include mainly strains of *L. acidophilus* and *Bifidobacterium* spp. and some of them are listed in Table 10.3.

The probiotic strains are mainly used as adjunct cultures because of their slow growth in milk (Shah, 2004).

Genus *Lactobacillus*

L. acidophilus is one of the most important probiotic species. It was isolated by Moro in 1900, who called it *B. acidophilus* due to its unusual growth in the acid environment. This species contains mainly obligately homofermenters with lactic acid as the major end product; however, a few are facultative heterofermenters. They occur naturally in the gastrointestinal tract of humans and animals, in the human mouth and vagina, and in some traditional

Table 10.3. Some of Probiotic Strains Used in Commercial Applications

Strains	Source
<i>L. acidophilus</i> LA1/LA5	Chr. Hansen
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> Lb12	
<i>L. paracasei</i> CRL431	
<i>B. animalis</i> ssp. <i>lactis</i> Bb12	Danisco
<i>L. acidophilus</i> NCFM®	
<i>L. acidophilus</i> La-14	
<i>L. paracasei</i> Lpc-37	
<i>B. lactis</i> HOWARU™/BI-04	DSM Food Specialties
<i>L. acidophilus</i> LAFTI® L10	
<i>B. lactis</i> LAFTI® B94	
<i>L. paracasei</i> LAFTI® L26	
<i>L. johnsonii</i> La1	Nestle
<i>L. acidophilus</i> SBT-20621	Snow Brand Milk Products Co., Ltd.
<i>B. longum</i> SBT-29281	
<i>L. rhamnosus</i> R0011	Institute Rosell
<i>L. acidophilus</i> R0052	
<i>L. casei</i> Shirota	Yakult
<i>B. breve</i> strain Yakult	
<i>B. lactis</i> HN019 (DR10)	Fonetera
<i>L. rhamnosus</i> HN001 (DR20)	
<i>L. plantarum</i> 299V	Probi AB
<i>L. rhamnosus</i> 271	
<i>L. fermentum</i> RC-14	Urex Biotech
<i>L. rhamnosus</i> GR-1	
<i>L. casei</i> Immunitas	Danone
<i>B. animalis</i> DN173010 (Bioactiva)	
<i>L. rhamnosus</i> LB21	Essum AB
<i>Lactococcus lactis</i> L1A	
<i>L. reuteri</i> SD2112	Biogaia
<i>L. rhamnosus</i> GG1	Valio Dairy
<i>L. salivarius</i> UCC118	University College Cork
<i>B. longum</i> BB536	Morinaga Milk Industry Co. Ltd.
<i>L. acidophilus</i> LB	Lacteol Laboratory
<i>L. paracasei</i> F19	Medipharm

Adapted from Holm (2003) and Shah (2004).

fermented dairy products, such as kefi. They are either microaerophilic, aerotolerant, or anaerobic and strictly fermentative with the G + C content of their DNA usually between 32 and 53 mol%.

L. acidophilus is a Gram-positive rod with rounded ends that occurs as single cells as well as in pairs or in short chains. Because of their microaerophilic nature, the surface growth on solid media is generally enhanced by anaerobic condition or reduced oxygen pressure. The organisms require carbohydrates as energy and carbon source as well as nucleotides, amino acids, and vitamins. *L. acidophilus* utilizes sucrose more effectively than do lactose. The optimum growth temperature of *L. acidophilus* is between 35 and 40°C, although the growth occurs at as high as 45°C. The acid tolerance varies from 0.3 to 1.9% titratable acidity, with an optimum growth at pH 5.5–6.0 (Shah, 2000). Because of low content of available peptides and amino acids in milk, *L. acidophilus* tends to grow slowly in this medium.

Genus *Bifidobacteriu*

Bifidobacteri were first isolated and visualized by Tissier in 1900 from feces of breast-fed infants. These rod-shaped, non-gas producing, and anaerobic organisms were named as *B. bifidu* due to their bifurcated morphology. They are generally characterized as Gram-positive, nonspore forming, nonmotile, and catalase-negative anaerobes with a special metabolic pathway, which allows them to produce acetic acid in addition to lactic acid in the molar ratio of 3:2. They are fastidious organisms and have special nutritional requirements, thus these bacteria are often difficult to isolate and grow in the laboratory (Shah, 2000). Because of their high (>50 mol%) G + C content, bifidobacteri are phylogenetically assigned in the actinomycete division of the Gram-positive bacteria. At present, there are 32 species in the genus *Bifidobacteriu*, 12 of which are isolated from human sources (i.e., dental caries, feces, and vagina), 15 from animal intestinal tracts or rumen, 3 from honeybees, and remaining 2 found in fermented milk and sewage (Shah and Lankaputhra, 2002).

Bifidobacteri are saccharolytic organisms and produce acetic acid and lactic acid without generating CO₂. The optimum growth pH is between 6.0 and 7.0, with no growth occurring at pH 4.5–5.0 or below or above pH 8.0. The strains of this species have the mesophilic optimum growth temperature in the range of 37–41°C, with the maximum at 43–45°C and minimum between 25 and 28°C.

SELECTION OF PROBIOTICS

In order to observe an appreciable probiotic effect, certain technological and physiological characteristics of probiotic strains are important. Although numerous criteria have been recognized and suggested (Mattila-Sandholm et al., 2002; Ouwehand et al., 1999; Reid, 1999), a general agreement exists in regard to key selection criteria listed in Table 10.4 (Morelli, 2007).

The first step in the selection of a probiotic is the determination of its taxonomic classification which may give an indication of the origin, habitat, and physiology of the strain. All these characteristics have important consequences on the selection of the novel strains. The classification and relatedness of probiotics (and other microorganisms) is based on the comparison of highly conserved regions of ribosomal RNA (rRNA). Many authors (i.e., Ouwehand et al., 1999) advocated the importance of origin in specific commercial applications. More recently, FAO/WHO (2001) expert panel suggested that the specificity of probiotic action is more important than the source of microorganism. Reports on the occurrence of harmful effects associated with consumption of probiotics are quite rare, although certain *Lactobacillus* strains have been isolated from bloodstream and local infections (Ishibashi and Yamazaki, 2001). Another important safety aspect is the antibiotic resistance of probiotics, since antibiotic resistant genes, especially those encoded by plasmids, could be transferred between microorganisms. The risk of gene transfer depends on the nature of the genetic material (plasmid, transposons), the nature and concentrations of the donor and recipient strains and their interactions, and the environmental conditions, that is, the presence of an antibiotic may facilitate the growth of antibiotic resistant mutants (Marteau, 2001). Therefore, the probiotic strains need to be tested for their natural antibiotic resistance to prevent the undesirable transfer of resistance to other endogenous bacteria.

VIABILITY OF PROBIOTICS

The viability and activity of probiotics in the products have been frequently cited as a prerequisite for achieving numerous beneficial health benefits. However, even nonviable cultures may exert certain functional properties such as immunomodulation (Ouwehand et al., 1999). Moreover, no general agreement has been reached on the recommended levels and the suggested levels ranged from 10⁶ CFU/mL (Kurmann and Rasic, 1991) to over 10⁷ and 10⁸

Table 10.4. Key and Desirable Criteria for the Selection of Probiotics in Commercial Applications

General	Property
Safety criteria	Origin
	Pathogenicity and infectivity
	Virulence factors—toxicity, metabolic activity, and intrinsic properties, that is, antibiotic resistance
Technological criteria	Genetically stable strains
	Desired viability during processing and storage
	Good sensory properties
Functional criteria	Phage resistance
	Large-scale production
	Tolerance to gastric acid and juices
	Bile tolerance
	Adhesion to mucosal surface
Desirable physiological criteria	Validated and documented health effects
	Immunomodulation
	Antagonistic activity toward gastrointestinal pathogens, that is, <i>Helicobacter pylori</i> , <i>Candida albicans</i>
	Cholesterol metabolism
	Lactose metabolism
	Antimutagenic and anticarcinogenic properties

Adapted from Morelli (2007).

CFU/mL (Lourens-Hattingh and Viljoen, 2001). In Japan, the Fermented Milks and Lactic Acid Bacteria Beverages Association has advocated an approach in which at least 10⁷ viable bifidobacteri per gram of a product is required to constitute a probiotic food for humans (Ishibashi and Shimamura, 1993). Viability and activity of the bacteria are important

Table 10.5. Different Stress Vectors Affecting Viability of Probiotic during Processing

Processing Step	Stress Vector
Production of probiotic preparations	Presence of organic acids during cultivation
	Concentration—high osmotic pressure, low water activity, and higher concentration of particular ions
	Temperature—freezing, vacuum, and spray drying
Production of a probiotic containing product	Drying
	Prolonged storage—oxygen exposure and temperature fluctuatio
	Nutrient depletion
	Strain antagonism
	Increased acidity
Gastrointestinal transit	Positive redox potential (presence of oxygen)
	Presence of antimicrobial compounds, that is, hydrogen peroxide and bacteriocins
	Storage temperature
	Gastric acid and juices
	Bile salts
	Microbial antagonism

considerations, because these bacteria must survive in the food during shelf life, during transit through the acidic conditions of the stomach, and resist degradation by hydrolytic enzymes and bile salts in the small intestine.

The viability and activity of probiotic cultures may be affected during all steps involved in a delivery process through the exposure to different stress vectors (Table 10.5).

In general, probiotics are extremely susceptible to environmental conditions such as water activity, positive redox potential, elevated temperature, and acidity (Siuta-Cruce and Goulet, 2001). In the initial phase, probiotic cultures are selected not only on the basis

of the functional criteria but also additional technological aspects including enhanced yields during cultivation at the industrial scale and improved survival during culture concentration and freeze drying. Furthermore, the viability of probiotics in a delivery system depends on a strain, interactions between species present, production of hydrogen peroxide due to bacterial metabolism, and final acidity of the product. Additionally, the viability would be also affected the availability of nutrients, growth promoters and inhibitors, concentration of sugars, dissolved oxygen and oxygen permeation through package (especially for *Bifidobacterium* spp.), inoculation level, and fermentation time (Shah, 2000).

IMPROVEMENT OF THE VIABILITY OF PROBIOTICS

The physiological effects of probiotic strains are related to their therapeutic levels and their viability and metabolically activity should be expressed into the gastrointestinal tract. The appropriate culture selection for the survival and maintenance of metabolic activity under various processing and physiological conditions especially acid environment of the stomach and bile salts is of primary interest. The tolerance to acids and bile appeared to be strain specific (Shah, 2000). Many strains of *L. acidophilus* and *Bifidobacterium* spp. inherently lack the ability to survive harsh conditions in the gut. Certain strains of *L. acidophilus* thrive well under acidic conditions and bile concentrations, while *B. longum* and *B. pseudolongum* showed best tolerance to acid and bile (Lankaputhra and Shah, 1995). The starter antagonism also can negatively affect the growth of probiotic strains due to the production of inhibitory compounds (Vinderola et al., 2002). On the contrary, starter cultures with a proteolytic or oxygen scavenging ability may be beneficial for the growth of bifidobacteria (Ishibashi and Shimamura, 1993). The final pH of the product appears to be the most crucial factor for the survival of probiotic organisms. Below pH 4.4, probiotics do not thrive well and a substantial decrease in number of probiotic bacteria is usually observed. Most frequently, this problem is avoided by increasing the level of inoculation (Dave and Shah, 1997) or the omission of certain starter strains (Donkor et al., 2006). Alternatively, probiotic strains may be added to the product post-fermentation.

The viability of probiotics in the product and subsequently in the gastrointestinal tract can be improved by addition of an appropriate prebiotic. Prebiotics are

defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that have the potential to improve health” (Gibson and Roberfroid, 1995). While their role by definition is the selective stimulation of a limited number of colonic and preferable beneficial bacteria, a range of prebiotics has been used as a tool for improvement of probiotic activity and survival in fermented foods during growth and storage (Bruno et al., 2002; Liong and Shah, 2005).

HEALTH POTENTIAL OF PROBIOTIC DAIRY PRODUCTS

PROBIOTIC EFFECT

A number of health benefits have been attributed to products containing probiotic organisms. While some of these benefits have been well documented and established, others have shown a promising potential in animal models. More importantly, health benefits imparted by probiotic bacteria are very strain specific; therefore, there is no universal strain that would provide all proposed benefits, not even strains of the same species. Moreover, not all the strains of the same species are effective against defined health conditions. The strains *L. rhamnosus* GG (Valio), *S. cerevisiae* Boulardii (Biocodex), *L. casei* Shirota (Yakult), and *B. animalis* Bb-12 (Chr. Hansen) are certainly the most investigated probiotic cultures with the established human health efficacy data against the management of lactose malabsorption, rotaviral diarrhea, antibiotic-associated diarrhea, and *Clostridium difficile* diarrhea. These strain-specific effects are listed in Table 10.6.

ALLEVIATION OF LACTOSE INTOLERANCE

The decline of the intestinal β -galactosidase (β -gal or commonly known as lactase) activity is a biological characteristic of the maturing intestine in the majority of the world's population. With the exception of the inhabitants of Northern and Central Europe and Caucasians in North America and Australia, over 70% of adults are lactose malabsorbers worldwide (de Vrese et al., 2001). Lactose upon ingestion is hydrolyzed by lactase in the brush border membrane of the mucosa of the small intestine into constitutive monosaccharides, glucose, and galactose, which are readily absorbed in the blood stream. However, the activity of the intestinal lactase in the lactose intolerant

Table 10.6. Some of the Established and Potential Health Benefits of Probiotic Organisms

Health Effect	Mechanism
	Scientificall established
Alleviation of lactose intolerance	Delivery of intracellular β -galactosidase into human gastrointestinal tract
Prevention and reduction of symptoms of rotavirus and antibiotic-associated diarrhea	Competitive exclusion Translocation/barrier effect Improved immune response
	Potential
Treatment and prevention of allergy (atopic eczema and food allergy)	Translocation/barrier effect Immune exclusion, elimination, and regulation
Reduction of risk associated with mutagenicity and carcinogenicity	Metabolism of mutagens Alteration of intestinal microecology Alteration of intestinal metabolic activity Normalization of intestinal permeability Enhanced intestinal immunity
Hypocholesterolemic effect	Deconjugation of bile salts
Inhibition of <i>Helicobacter pylori</i> and intestinal pathogens	Competitive exclusion Barrier effect Production of antimicrobial compounds
Prevention of inflammator bowel diseases	Competitive exclusion Improvement of epithelial tight junctions Modificatio of intestinal permeability Modulation of immune response Production of antimicrobial products Decomposition of pathogenic antigens
Stimulation of immune system	Recognition by toll-like receptors—induction of innate and adaptive immunity; Downregulation of pro-inflammatory cytokines and chemokines Upregulation of phagocytic activity Regulation of Th1/Th2 balance

individuals is usually less than 10% of childhood levels (Buller and Grand, 1990). This decline, termed hypolactasia, causes insufficient lactose digestion in the small intestine, characterized by an increase in blood glucose concentration or hydrogen concentration in breath upon ingestion of 50 grams lactose, conditions designated as lactose maldigestion (Scrimshaw and Murray, 1988). Hypolactasia and lactose malabsorption accompanied with clinical symptoms, such as bloating, flatulence nausea, abdominal pain, and diarrhea, are termed lactose intolerance. Symptoms are caused by undigested lactose in the large intestine, where lactose is fermented by intestinal microflor and osmotically increases the water fl w into the lumen. The severity of the

symptoms depends primarily on the size of the lactose load ingested. The development of the intolerance symptoms also depends on the rate of lactose transit to the large intestine, influence by the osmotic and caloric load, and the ability of the colonic microflor to ferment lactose (Martini and Savaiano, 1988).

Numerous studies have shown that individuals with hypolactasia could tolerate fermented dairy products better than an equivalent quantity in milk (Hertzler and Clancy, 2003; Montalto et al., 2005). At least three factors appear to be responsible for a better tolerance of lactose in fermented milk including (a) starter culture, (b) intracellular enzyme β -galactosidase or lactase expressed in these cultures,

and (c) oro-caecal transit time. The traditional cultures used in dairy fermentations utilize lactose as an energy source during growth, thus, at least partially reducing its content in fermented products. The bacterial lactase may escape the luminal denaturation which may lead to lactose hydrolysis and improved lactose tolerance. The increased viscosity of fermented milk, in this case yogurt, may also slow down the gastric emptying and consequently prolong the transit time through the gastrointestinal tract improving absorption of lactose and lactose tolerance.

PREVENTION AND REDUCTION OF DIARRHEA SYMPTOMS

One of the main applications of probiotics has been the treatment and prevention of antibiotic-associated diarrhea, which is often caused by occurrence of *C. difficile* after an antibiotic treatment. *C. difficile* is an indigenous gastrointestinal organism usually encountered in low numbers in the healthy intestine; however, the antibiotic treatment may lead to a disruption of indigenous microflora and subsequently to an increase in the concentration of this organism and toxin production, which causes symptoms of diarrhea. The administration of an exogenous probiotic preparation is required to restore the balance of the intestinal microflora (Sazawal et al., 2006). The strongest evidence of a beneficial effect of defined strains of probiotics has been established for *L. rhamnosus* GG and *B. animalis* Bb-12. Administration of oral rehydration solution containing *Lactobacillus* GG to children with acute diarrhea resulted in a reduction of the duration of diarrhea, lower chance of a protracted course, and faster discharge from the hospital (Guandalini et al., 2000). Similar to antibiotic- and rotavirus-associated diarrhea, probiotics may prevent and alleviate symptoms of traveller's diarrhea, which is caused by bacteria, particularly enterotoxigenic *Escherichia coli*. Several studies have assessed the effects of probiotic preparations as prophylaxis for traveller's diarrhea; however, the results have been conflicting due to methodological deficiencies, which certainly limited the validity of their conclusions (Marteau et al., 2002).

The mechanisms by which probiotic cultured dairy foods reduce the duration of diarrhea are still largely unknown. Several possible mechanisms are listed in Table 10.6. A competitive exclusion is the mechanism by which probiotics inhibit the adhesion of rotavirus by modifying the glycosylation state of

the receptor in epithelial cells via excreted soluble factors (Freitas et al., 2003). The presence of probiotics also prevents the disruption of the cytoskeletal proteins in the epithelial cells caused by the pathogen, which leads to the improved mucosal barrier function and prevention of the failure in the secretion of electrolytes (Resta-Lenert and Barrett, 2003). Additionally, probiotic strains may modulate the innate immune response both to anti- and pro-inflammatory directions (Baat et al., 2004).

TREATMENT AND PREVENTION OF ALLERGY

The prevention and management of allergies is one of the areas in which probiotics may potentially exert their beneficial role. A delayed colonization of *Bifidobacterium* and *Lactobacillus* spp. in the gastrointestinal tract of children may be one of the reasons for allergic reactions (Kalliomäki and Isolauri, 2003). Early consumption of probiotic preparations containing *Lactobacillus* GG may reduce prevalence of atopic eczema later in life (Gueimonde et al., 2006). Similarly, the treatment with *Lactobacillus* GG may alleviate atopic eczema/dermatitis syndrome symptoms in IgE-sensitized infants but not in non-IgE-sensitized infants (Viljanen et al., 2005a), while a 4-week treatment with *Lactobacillus* GG alleviated intestinal inflammation in infants with atopic eczema/dermatitis syndrome and milk allergy (Viljanen et al., 2005b). The mechanisms of the protective effects of probiotics on allergic reactions are not known, although the reinforcement of the different lines of gut defense including immune exclusion, immune elimination, and immune regulation has been suggested (Isolauri et al., 2005).

REDUCTION OF THE RISK ASSOCIATED WITH MUTAGENICITY AND CARCINOGENICITY

Antigenotoxicity, antimutagenicity, and anticarcinogenicity are important potential functional properties of probiotics. Mutagens are frequently formed during stress, or due to viral or bacterial infections and phagocytosis. Endogenous DNA damage is one of the contributors to ageing and age-related degenerative diseases. The defense mechanism via leukocytes liberates a range of compounds including NO, O₂⁻, and H₂O₂ thus defending an individual from bacterial and viral infections, but these may contribute to DNA damage and mutations. The DNA irreversible

damage is a critical factor of carcinogenesis and ageing. Antimutagenicity could be described as a suppression of the mutation process, which manifests itself as a decrease in the level of spontaneous and induced mutations. The probiotic intake may be related to a reduced colon cancer incidence (Hirayama and Rafter, 2000), and experimental studies showed the ability of lactobacilli and bifidobacteria to decrease the genotoxic activity of certain chemical compounds (Tavan et al., 2002) and increase in antimutagenic activity during the growth in selected media (Lo et al., 2004).

Several factors have been identified to be responsible for induction of colorectal cancer including bacteria and metabolic products such as genotoxic compounds (nitrosamine, heterocyclic amines, phenolic compounds, and ammonia). The diet plays a role in the etiology of most large bowel cancers, thus it is a potentially preventable disease. Many studies have confirmed the involvement of the endogenous microflora in the onset of colon cancer. This effect is mediated by microbial enzymes such as β -glucuronidase, azoreductase, and nitroreductase, which convert procarcinogens into carcinogens (Goldin and Gorbach, 1984). Several studies have shown that the preparation containing LAB inhibits the growth of tumor cells in experimental animals or indirectly lowers carcinogenicity by decreasing bacterial enzymes that activate carcinogenesis (Rafter, 2002). Short-chain fatty acids produced by *L. acidophilus* and bifidobacteria were also reported to inhibit the generation of carcinogenic products by reducing enzyme activities. When incubated in vitro with 4-nitroquinoline-1-oxide (4NQO), some probiotic strains inhibited the genotoxic activity of 4NQO. *L. casei* was most effective, followed by *L. plantarum* and *L. rhamnosus* (Cenci et al., 2002). The most convincing clinical data exist for *L. casei* strain Shirota in which the consumption of this organism was associated with the decreased urinary mutagen excretion. Furthermore, the habitual consumption of the fermented milk with this strain reduced the risk of bladder cancer in the Japanese population (Ohashi, 2000).

The mechanism of antimutagenicity and anticarcinogenicity of probiotic bacteria has not been clearly understood. The microbial binding of mutagens to the cell surface could be a possible mechanism of antimutagenicity (Orrhage et al., 1994). Other proposed mechanisms include alteration of intestinal microecology and intestinal metabolic activity, normalization of intestinal permeability, and enhanced intestinal immunity (Shah, 2007).

HYPCHOLESTEROLEMIC EFFECT

Diet rich in saturated fat or cholesterol leads to an increase in the serum cholesterol level, which is one of the major factors for coronary heart diseases. Elevated levels of serum cholesterol, particularly LDL-cholesterol, have been linked to an increased risk for cardiovascular disease. Mann and Spoerry (1974) were the first to observe a decrease in the serum cholesterol levels in men fed large quantities (8.33 liter per man per day) of milk fermented with *Lactobacillus*. This was possibly due to the production of hydroxymethyl-glutarate by probiotic bacteria, which is reported to inhibit hydroxymethylglutaryl-CoA reductases required for the synthesis of cholesterol. However, this has not been substantiated with human studies, and results were rather contradictory with studies reporting either lowering effect (Agerholm-Larsen et al., 2000) or no effect was observed (De Roos et al., 1999; Lewis and Burmeister, 2005), even though in the latter the strains were able to reduce cholesterol in vitro. Probiotic bacteria are reported to deconjugate bile salts, which leads to a reduction in cholesterol level. *L. acidophilus* is also reported to take up cholesterol during growth and this makes it unavailable for absorption into the blood stream (Shah, 2006).

INHIBITION OF *Helicobacter pylori* AND INTESTINAL PATHOGENS

Probiotic cultures produce a wide range of antibacterial compounds including organic acids (e.g., lactic acid and acetic acid), hydrogen peroxide, bacteriocins, various low-molecular-mass peptides, and antifungal peptides/proteins, fatty acids, phenyllactic acid, and OH-phenyllactic acid. Lactic and acetic acids are the main organic acids produced during the growth of probiotics, accounting for over 90% of the acids produced. Lowering of pH due to lactic or acetic acid produced by these bacteria in the gastrointestinal tract has a bacteriocidal or bacteriostatic effect. Moreover, a heat-stable, low-molecular-weight antibacterial substance was present in the cell-free culture supernatant resulting in the inactivation of a wide range of Gram-negative bacteria and inhibition of the adhesion to and invasion of Caco-2 cells by *Salmonella enterica* ser. *typhimurium* (Coconnier et al., 2000; Liévin-Le Moal et al., 2002). In some instances, this inhibition is multifactorial including all mentioned factors (Fayol-Messaoudi et al., 2005). The production of these antimicrobial compounds

appeared to be stimulated by the presence of pathogens (Rossland et al., 2005). In general, many mechanisms have been suggested by which probiotics prevent the detrimental effect of the intestinal pathogens including competition for limited nutrients, inhibition of the epithelial and mucosal adherence of pathogens, inhibition of epithelial invasion by pathogens, the production of antimicrobial substances, and/or the stimulation of mucosal immunity.

H. pylori is an intestinal pathogen, which causes peptic ulcers, type B gastritis, and chronic gastritis. It resides in the stomach as an opportunistic pathogen without causing any symptoms. An increased density of *H. pylori* on the gastric mucosa is associated with more severe gastritis and an increased incidence of peptic ulcers. One of the measures which may help reduce rate of infection is a diet modulation with the inclusion of probiotics (Khulusi et al., 1995). Antibiotic treatments are successfully used to eradicate *H. pylori*. However, some side effects are usually encountered including antibiotic-associated diarrhea and likelihood of induction of the antibiotic resistance in the intestinal pathogens. Probiotic organisms do not appear to eradicate *H. pylori*, but they are able to reduce the bacterial load in patients infected with *H. pylori*. *Lactobacillus johnsonii* La1 and *L. gasseri* OLL2716 have been found to reduce *H. pylori* colonization and inflammation (Felley et al., 2001). Similarly, *L. casei* Shirota and *L. acidophilus* were able to inhibit the growth of *H. pylori*. In an intervention study, 14 patients infected with *H. pylori* received *L. casei* Shirota (2×10^{10} CFU per day) fermented milk for 6 weeks. Ureolytic activity was reduced in 64% of the patients who consumed fermented products containing probiotics, compared to 33% of the control group (Cats et al., 2003). Several mechanisms in regard to the effect of probiotics on *H. pylori* have been suggested including production of antimicrobial substances, enhanced gut barrier function, and competition for adhesion sites; however, the relative importance of these mechanisms is still unclear.

PREVENTION OF INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease) may be related to the alterations in the intestinal microflora. Immunological upregulated T-helper (Th) 1 cell response and failure of the mucosal tolerance against the indigenous microflora have been indicated as a possible

cause in various forms of IBD (Xavier and Podolsky, 2000). At present, IBD affects up to 2 million people worldwide. It is characterized by symptoms that include a disturbance in bowel habits and mucosal inflammation. The intestinal microflora in IBD patients loses the anti-inflammatory function that existed in normal condition, followed with a reduction in the number of anaerobic bacteria and lactobacillus. The administration of probiotics may help restore microbial homeostasis in the gut, downregulate intestinal inflammation and ameliorate the diseases; however, the effect appears to be a strain specific (Schultz et al., 2003).

STIMULATION OF IMMUNE SYSTEM

The human body is constantly exposed to various external stresses including viruses, bacteria, and parasites. Therefore, the critical role of the immune system, which, in principle, plays a major role in the defense line, becomes clinically apparent when the protection fails. The inherited and acquired immunodeficiency can be described as an increased susceptibility to infections, sometimes caused by indigenous intestinal microorganisms that are not normally considered to be pathogenic. Human immune system is divided into innate (natural or nonspecific) and adaptive (acquired or specific) immunity. Humans as mammals have developed an extremely sophisticated adaptive immune system of both systemic and mucosal (local) type. Intestinal epithelial cells are in direct contact with the intestinal microflora and also interface and segregate the immune system. It is suggested that the immune system might be beneficially affected in the presence of probiotics through the action of recognition receptors expressed on the surface of epithelial cells. The innate immune system via toll-like receptors (TLRs) recognizes a large group of chemical structures in pathogens such as lipopolysaccharides (LPS) and lipoteichoic acids which enables them to recognize foreign objects which trigger a cascade of immunological defense mechanisms, such as the production of pro- and anti-inflammatory cytokines (Anderson, 2000). TLRs are not only expressed mainly by macrophages and dendritic cells (DCs) but also include a variety of other cell types such as B cells and epithelial cells (Pasare and Medzhitov, 2005). The activation of TLRs results in the initiation of the response of the DCs which leads to the production of cytokines and upregulation or downregulation of cell-surface molecules (Granucci and Ricciardi-Castagnoli, 2003). These

signals critically influence further induction of both innate and adaptive immunity.

The suppression of the formation of pro-inflammatory cytokines and chemokines in the presence of probiotics has been reported in several *in vitro* studies. The response of the immune system to a probiotic was weaker than in the presence of a Gram-positive pathogen. More importantly, human monocyte-derived DCs responded differently to different Gram-positive bacteria (Veckman et al., 2004). The different immune response to various bacteria was confirmed in another study in which Gram-negative *Klebsiella pneumoniae* and *L. rhamnosus* were compared (Braat et al., 2004). Both cultures induced DC maturation but resulted in a different cytokine profile. *K. pneumoniae* activated the expression of Th1-type cells, where *L. rhamnosus* reduced the production of the pro-inflammatory cytokines (TNF- α) and interleukins (IL-6 and IL-12) by immature DCs and the production of IL-12 and IL-18 by mature DCs. Moreover, the cytokine response may vary greatly in the presence of different probiotics. The mixture of eight different probiotic and LAB strains including *L. acidophilus*, *L. delbrueckii* ssp. *bulgaricus*, *L. casei*, *L. plantarum*, *B. longum*, *B. infantis*, *B. breve*, and *S. thermophilus* upregulated the production of IL-10 and downregulated the pro-

duction of IL-12 by DCs derived from human blood and lamina propria. The pro-inflammatory effect was reduced by suppression of IL-12 production in the presence of the probiotics, while maintaining high production of IL-10, which was regulated by bifidobacteria that upregulated IL-10 production. Furthermore, most of the strains suppressed IL-12 production (Hart et al., 2004; Lammers et al., 2003).

QUALITY CONTROL OF CULTURED DAIRY PRODUCTS

The quality of the final product should comply with applicable standards in regard to its chemical, physical, microbiological, and nutritional properties. The ultimate test of the product quality is the consumers' acceptance. The product foremost must be safe for human consumption and conform to any regulations imposed by the regulatory bodies. In addition, it should maintain high sensory and quality standards throughout its shelf life. These attributes are generally achieved by using two intertwined concepts: good manufacturing practice (GMP) and hazard analysis critical control points (HACCP). Furthermore, the chemical microbial analysis of raw materials is extremely important since they govern the quality of the final product.

Table 10.7. Recommended Media for Selective Enumeration of *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, *L. acidophilus*, *Bifidobacterium* spp., *L. casei*, *L. rhamnosus*, and Propionibacteria in a Mixture of Bacteria

Agar	Bacteria	Incubation Conditions	Colony Morphology
S. thermophilus agar	<i>S. thermophilus</i>	Aerobic, 37°C, 24 hours	0.1–0.5 mm, round yellowish
MRS ^a agar (pH 4.58)	<i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	Anaerobic, 45°C, 72 hours	1.0 mm, white, cottony, rough, irregular
MRS-sorbitol agar	<i>L. acidophilus</i>	Anaerobic, 37°C, 72 hours	Rough, dull, small (0.1–0.5), brownish
MRS-NNLP ^b agar	Bifidobacteri	Anaerobic, 37°C, 72 hours	1 mm, white, smooth, shiny
MRS-vancomycin agar ^c	<i>L. casei</i>	Anaerobic, 37°C, 72 hours	1.0 mm, white shiny, smooth
MRS-vancomycin agar	<i>L. rhamnosus</i>	Anaerobic, 43°C, 72 hours	1.0–2.0 mm, white shiny, smooth
Sodium lactate agar	Propionibacteria ⁴	Anaerobic, 30°C, 7–9 days	1.0–2.5 mm, dull brown, lighter margin

^a de man, Rogosa, and Sharpe agar.
^b Nalidixic acid, neomycin sulfate, lithium chloride, and paromomycin sulfate.
^c In case *L. rhamnosus* absent; if not, then subtraction method required.
Adapted from Tharmaraj and Shah (2003).

Inclusion of different cultures especially probiotics imposes additional requirement. As previously stated, probiotics need to be delivered in an active and viable form at a desired therapeutic level. However, probiotic strains grow poorly in milk, resulting in low final concentrations and even the loss of the viability during prolonged cold storage. As an example, a number of commercial products of yogurts were analyzed in Australia and Europe for the presence of *L. acidophilus* and *Bifidobacterium* over the years (Iwana et al., 1993; Micanel et al., 1997; Shah et al., 1995; Tharmaraj and Shah, 2004; Vinderola et al., 2000). Most of the products contained variable if not very low concentrations of probiotics, especially bifidobacteria. Viability and activity of the bacteria are important considerations, because these bacteria must survive in the food during shelf life, during transit through the acidic conditions of the stomach, and resist degradation by hydrolytic enzymes and bile salts in the small intestine. Furthermore, adequate enumeration techniques are required in order to properly assess the viability and survival of probiotic bacteria, especially in the light of the labeling requirements. Several media for selective enumeration of *L. acidophilus*, *Bifidobacterium* spp., and *L. casei* were proposed in the 1990s; however, most of these methods were based on pure cultures of these organisms. Consequently, these methods were considered rather inaccurate (Talwalkar and Kailasapathy, 2004). More recently, Tharmaraj and Shah (2003) recommended media for selective enumeration of *St. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Bifidobacterium* spp., *Lb. casei*, *Lb. rhamnosus*, and propionibacteria in a mixture of probiotic bacteria. Their recommendations are summarized in Table 10.7.

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11

Butter and Spreads: Manufacture and Quality Assurance

Anna M. Fearon and Matthew Golding

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INTRODUCTION

Butter and dairy spreads are members of a group commonly identified as “yellow fat spreads.” The term also encompasses margarine and other products used for a similar purpose and which are emulsions of water and oil/fat derived from milk or other animal sources and/or plant sources. This chapter will concentrate on those yellow fat spreads described as “milk fat products” and which fall within the remit of the European Council (EC) Regulation 2991/94 relating to standards for “spreadable fats.” “Spreadable fats” are defined as having a fat content less than 90% but more than 10%, the fat content must represent at least two-thirds of the dry matter (excluding salt),

they should remain solid at room temperature (20°C) and are suitable for use as spreads. The sales descriptions of “spreadable fats” describe how the product should be identified to consumers and are detailed in the Annex of EC Council Regulation 2991/94. Such descriptions must include the origin of the fat (e.g., vegetable oil and/or milk fat and/or animal fat) and the total percentage fat content of the spread (see Table 11.1). There are three types of “spreadable fats” and these are categorized on the basis of the origin of the fat used:

- Milk fat products such as “butter” and “dairy spreads”;
- Vegetable and/or animal fat products such as “margarine” and “fat spreads”; and
- Mixed fat products (milk fat and vegetable/animal fats), such as “blends” and “blended spreads.”

Not all yellow fat spreads are termed “spreadable fats” although they may still be suitable for use as alternatives to butter and margarine. These “other yellow fat spreads” lie beyond the scope of the EC Council Regulation 2991/94 and include:

- Products with a fat content less than 10%;
- Products with a fat content of more than 90% (e.g., concentrated butter); and
- Mixed fat products with a milk fat content of more than 3% but less than 10% of the total fat.

Use of the term “butter” is also governed by the EC Council Regulation 2991/94 and EEC Council Regulation 1898/87 (protection of dairy designations). “Butter” is reserved for a product with a milk fat content of not less than 80% but less than 90%, a maximum water content of 16% and a maximum dry nonfat material content of 2%. The dairy designation

Table 11.1. EC Sales Descriptions of “Spreadable Fats”

Fat content ranges	Sales Descriptions		
	Milk fat products	Vegetable/animal fat products	Mixed milk fat and vegetable/animal fat
Equal or more than 80% but less than 90%	Butter	Margarine	Blend
More than 62% but less than 80%	Dairy spread <i>X</i> %	Fat spread <i>X</i> %	Blended spread <i>X</i> %
Equal to or more than 60% but less than or equal to 62%	Three-quarter fat butter Reduced fat butter	Three-quarter fat margarine Reduced fat margarine	Three-quarter fat blend Reduced fat blend
More than 41% but less than 60%	Dairy spread <i>X</i> % Reduced fat dairy spread <i>X</i> %	Fat spread <i>X</i> % Reduced fat spread <i>X</i> %	Blended spread <i>X</i> % Reduced fat blended spread <i>X</i> %
More than or equal to 39% but less than or equal to 41%	Half-fat butter Low-fat butter Light butter	Half-fat margarine Minerine Halverine Low-fat margarine Light margarine	Half-fat blend Low-fat blend Light blend
Less than 39%	Dairy spread <i>X</i> % Low-fat dairy spread <i>X</i> % Light dairy spread <i>X</i> %	Fat spread <i>X</i> % Low-fat spread <i>X</i> % Light fat spread <i>X</i> %	Blended spread <i>X</i> % Low-fat blend <i>X</i> % Light blend <i>X</i> %

Adapted from Annex of EC Council Regulation 2991/94.

regulations allow only milk fat as the fat source within such a product. Exceptions regarding use of the term “butter” are permitted where traditional usage of the term “butter” has applied to a characteristic of the product, for example, “peanut butter” and “cocoa butter” (EEC Council Regulation 1898/87). “Butter” may also be applied to composite products where the end product contains at least 75% milk fat and where an essential part of the end product, in terms of quantity or characterization, is butter. Regulations defining butter composition and labeling in other major butter-producing countries such as New Zealand and United States are similar to those described in the EC Council Regulations 1898/87 and 2991/94.

PRINCIPLES OF BUTTERMAKING

The main steps in the production of sweet cream butter are common whether carried out in a batch or continuous buttermaker and are summarized below:

- Preparation of cream by centrifugal separation of liquid milk to a fat content typically ca. 40%.
- Cream ageing to promote crystallization of milk fat using selected temperature regime(s).

- Emulsion destabilization and phase inversion from an oil/water cream emulsion to water/oil butter emulsion achieved by physical agitation (churning).
- Physical working of butter grains to form larger granules, expel buttermilk, distribute moisture, and create a homogeneous butter mass.

The following sections look more closely at the microstructural changes that occur during the butter-making process and how application of technology and choice of ingredients can help the manufacturer to optimize the process and product properties.

MICROSTRUCTURAL ASPECTS OF BUTTERMAKING

As with many manufactured foods, butter is an emulsion-based system. However, while most emulsion-based foods, such as milk, ice cream, dressings, mayonnaise, and yogurt are examples of oil-in-water systems, in which oil or fat droplets are dispersed in a continuous aqueous phase, butter is a water-in-oil-type emulsion, in which a relatively low volume fraction of water droplets are trapped within a partially crystalline fat matrix.

The fat content of the butter emulsion is typically >80%, based on a combination of quality, product stability, and legislative requirements. Water takes up approximately 15–18% of the final composition, and there are smaller quantities of salt and protein (each ca. 1%), and lipophilic vitamins. The presence of the dispersed aqueous phase containing the milk protein and salt is an important aspect in delivering the desired organoleptic properties, as will be discussed later. Consequently, the ability to create the required emulsion structure is key to producing a high-quality product.

To arrive at the final product requires a number of processing steps, as summarized in Figure 11.1, with each processing step integral in achieving the microstructural changes essential for delivering the required butter structure.

The buttermaking process starts with raw milk, an oil-in-water emulsion containing 3–4% protein, 4–5% carbohydrate, and 3–4% fat. Milk fat droplets in milk are typically in the range of 0.5–10 μm in diameter and contain 40% unsaturated triglycerides, 58% saturated triglycerides with the remainder being primarily interfacial material in the form of mono- and diglycerides, phospho- and glycolipids, and lipoproteins.

The milk emulsion itself is actually quite unstable against both coalescence and creaming and will show signs of separation after a few days storage. However, from a biological perspective, milk is intended for immediate consumption and so stability is relative. To prolong storage, most dairy milk is usually homogenized to reduce droplet size and improve both the creaming and coalescence stability.

SEPARATION AND COOLING

The first step of the buttermaking process requires the separation of the milk fat from the serum phase. Milk fat droplets are of course less dense than the aqueous phase, and therefore over time will tend to separate out due to creaming. Creaming rate is partly dependent on droplet size, and while larger droplets (2–10 μm) may cream over a few days, it can take considerably longer for smaller droplets (<2 μm). Early butter manufacturing relied on acidification through addition of cultures to assist in separating the cream (McDowall et al., 1960). Acidification causes precipitation of the proteins in the milk, a consequence of which is the entrapment of fat globules within the protein matrix. The resulting coagulum can then be more readily separated from the milk.

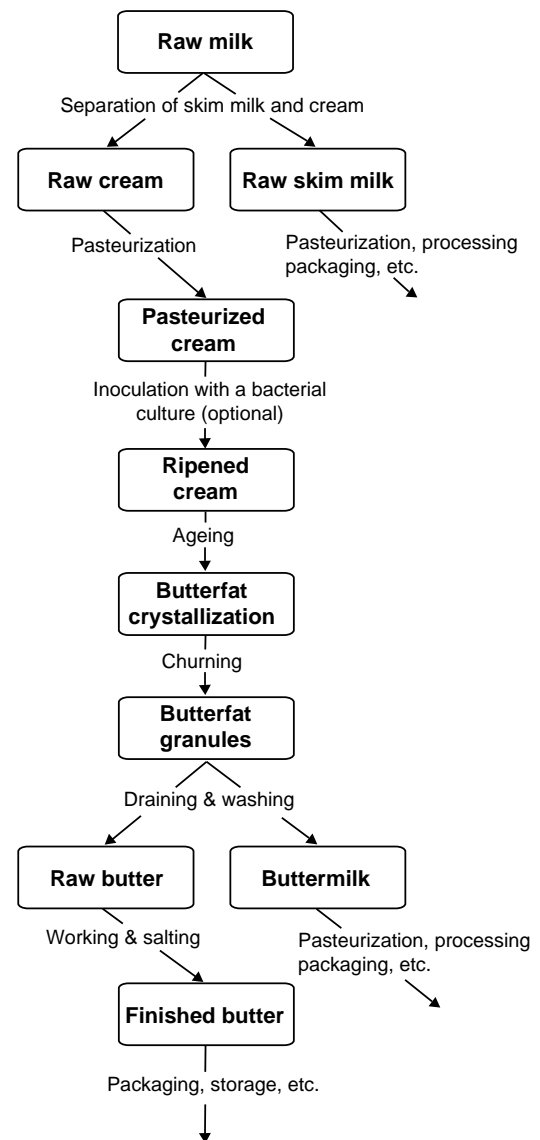


Figure 11.1. Key processing steps involved in the commercial manufacture of butter.

To achieve good cream separation in as short a time as possible, the creaming process can be accelerated using industrial centrifuges to produce cream fractions of at least 25% milk fat (the concentration of fat can vary depending on the particular processing route). After a pasteurization step, the cream is cooled and starter cultures may be added to lower the pH and develop flavor. The cooling step

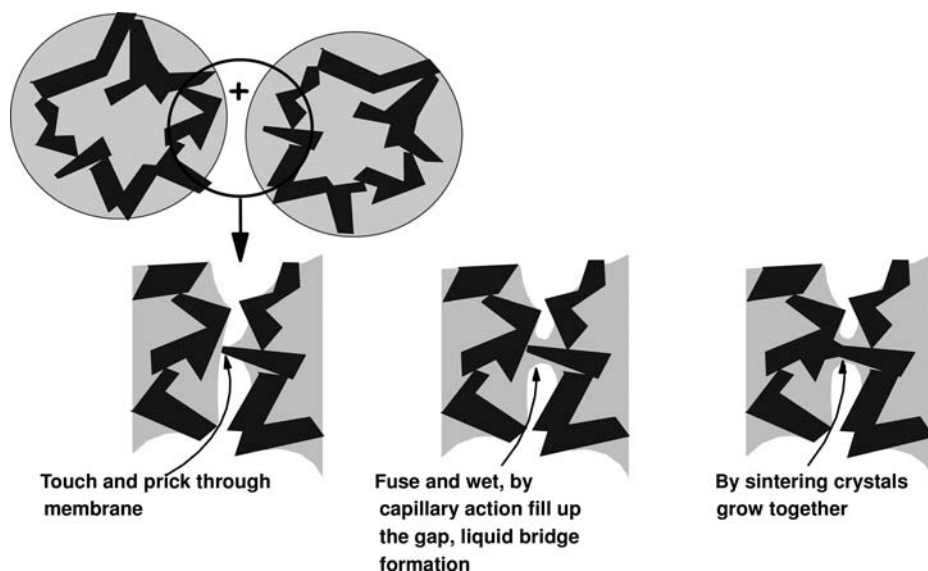


Figure 11.2. Schematic diagram showing the mechanistic step of interfacial rupture, wetting, fusion, and sintering that lead to the formation of partially coalesced droplets.

is essential to insuring that the butter has the right consistency. This is partly due to the fact that a prerequisite concentration of solid fat is necessary to develop butter grains during the churning process, and also partly due to the fact that the crystal structure of the fat can vary depending on thermal processing, with important consequences on the rheological properties of the finished product, as will be discussed.

CHURNING

After the initial cooling step the cream is transferred to an ageing tank where additional heat treatments may be applied to insure the most appropriate crystalline structure for the fat globules is reached. The ageing step may take up to 15 hours to insure that fat crystallization has reached equilibrium. After the ageing step the cream is transferred to the churn. Churning is the mechanical agitation of the cream at 10–15°C. Significant changes to the microstructure of the dairy emulsion take place during this process, as the emulsion undergoes a process of phase inversion from the initial cream oil-in-water-type emulsion to a water-in-oil-type emulsion.

The initial part of the inversion takes place through shear-mediated aggregation of fat droplets through a

process of partial coalescence. Partial coalescence requires that the droplet interfaces between colliding droplets are ruptured, leading to wetting, fusion, and sintering of droplets (see Fig. 11.2). In the case of full coalescence, the mechanism of instability is caused by rupture of the interface between two colliding droplets. In this case, the interior of the droplets is entirely liquid, and consequently collision and rupture of two approaching droplets will lead to the formation of a single larger combined droplet. In the case of partial coalescence, the droplets are semicrystalline, and consequently interfacial rupture and subsequent wetting of the oil phase will lead to the formation of fat agglomerates in which the integrity of the original droplet is partially maintained.

Limited partial coalescence is the structuring mechanism behind whipped dairy cream (of minimum 30% fat), in which a fat network of partially aggregated droplets is generated through the whipping process (see Fig. 11.3). This is responsible for stabilizing the foam structure, as well as providing increased viscosity and stand-up properties to the foam. As anyone who has made whipped cream will know, over-beating of the cream results in a collapse of the foam structure due to excessive fat agglomeration. However, this is an important part of the fat structuring needed to produce butter.

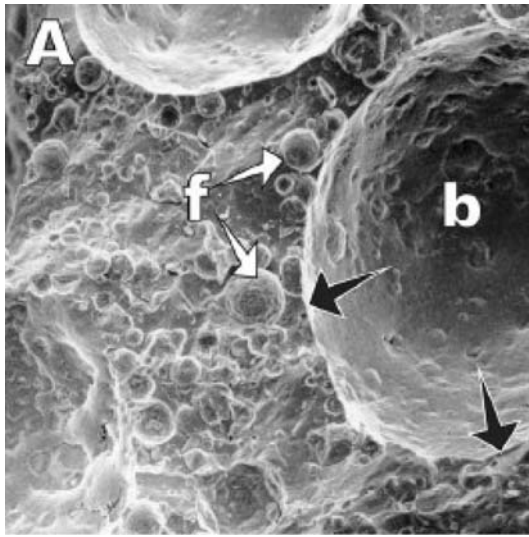


Figure 11.3. Scanning electron micrograph of development of a partially coalesced fat network, and extensive adsorption of fat globules to a bubble surface (Smith et al., 2000). “f” denotes fat globules and “b” denotes interior surface of an air bubble.

Research has shown that the partial coalescence of fat during the churning process is in fact greatly accelerated by the incorporation of air while beating, which is why air is incorporated as part of aerated (flotation churning (Frede and Buchheim, 1994). This has two effects on the coalescence process. Firstly, the incorporation of air into the emulsion greatly increases the local shear rate, leading to more frequent droplet collisions with greater force. Secondly, adsorption and wetting of fat droplets to the surface of bubbles during the churning process allows the spreading of liquid oil at the air–water interface. This spreading effect provides interconnectivity between droplets on the bubble surface, and once the bubble collapses, the droplets remain agglomerated leading to the formation of butter grains (see Fig. 11.4).

The ability of fat droplets to spread and adsorb onto a bubble surface is in part dependent on the presence of other surface-active components present in the formulation. For example, research carried out by Besner and Kessler (1998) has shown that the whipping time of creams could be controlled by the inclusion of different protein systems (see Fig. 11.5). The adsorption of protein to the air–water interface

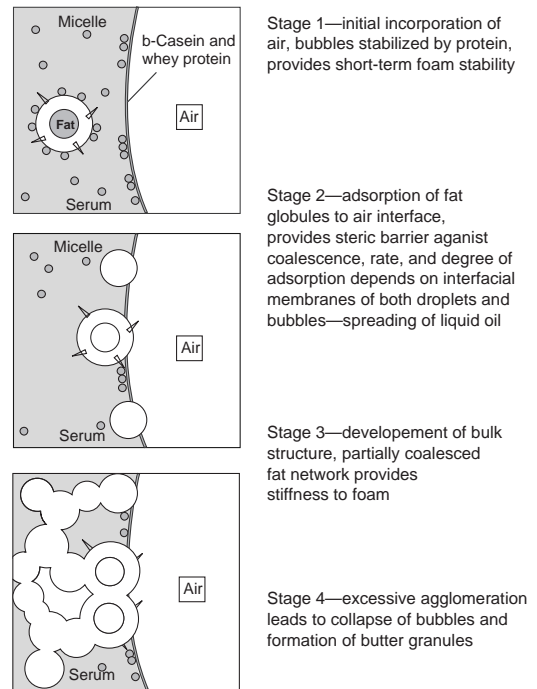


Figure 11.4. Schematic representation of formation of butter granules during flotation churning.

was seen to provide a barrier to droplet adsorption, with a consequent increase in whipping time being observed.

A comparison of the whipping properties of creams containing added fractions of casein and whey protein showed that the inclusion of whey protein, which formed a more viscoelastic air–water interface, resulted in the longest whipping time. In comparison, microparticulated whey, which was less effective at adsorbing to the air–water interface, resulted in a greatly reduced whipping time. Reducing the protein concentration present in butter creams prior to (aeration assisted) churning will therefore help to reduce the time needed to achieve butter grain formation, although it should be noted that some protein may be necessary to assist in the initial foam formation.

A second factor affecting fat agglomeration is the relative solid fat content of the emulsion droplets. A certain liquid content is required in order for spreading and wetting to take place, while a certain degree of solid fat is necessary to maintain rigidity of the butter granules. Consequently, shearing of high liquid oil emulsions may result in full coalescence and

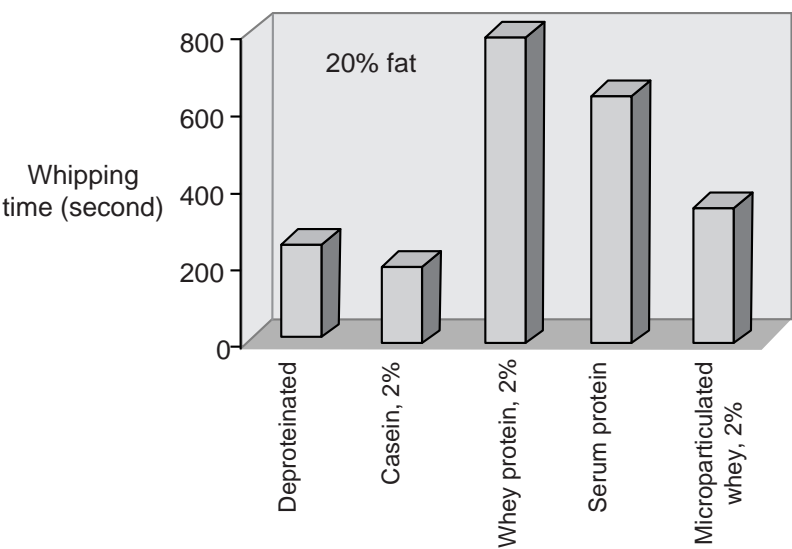


Figure 11.5. The effects of protein addition on the whipping time of nonhomogenized whipping creams.

phase separation of the emulsion, while droplets with very high solid fat contents may not agglomerate at all. Variations in solid fat content (and also the crystallinity of emulsion droplets) can have significant impact on the material properties of the final product. These properties can be affected either as a consequence of the particular triglyceride composition of the milk fat, or through the temperature treatment of the emulsion prior to churning, as well as the churning temperature itself (Schaffer et al., 1999).

The fatty acids of milk fat are typically composed as follows (by mass fraction) (see Table 11.2).

However, this composition is by no means fixed and can vary according to species, geography, climate,

and a number of other environmental factors (Shi et al., 2001). As a consequence of variation in milk fat composition, fats comprising higher levels of saturated fatty acids can result in firmer butters due to a higher solid fat content, while fats containing higher levels of unsaturated fatty acids (or those to which unsaturated vegetable oils have been added) may produce softer, more spreadable butters. Recent developments in milk fat fractionation have also allowed for additional control over triglyceride composition, again allowing the manufacture of butters with improved spreadability (Henning et al., 2006).

In addition to compositional effects, the solid fat content can be manipulated using appropriate temperature treatment (Bornaz et al., 1995). After pasteurization, emulsion droplets will be completely liquid in composition. However, during subsequent cooling of the cream a proportion of the fat will crystallize (in part dependent on fatty acid composition, as discussed). If cooling is rapid, there will be extensive nucleation of the fat, leading to the formation of many, small crystals. However, a slow cooling process will result in fewer nucleation sites thereby yielding fewer, but larger crystals.

Generally, increasing the rate of cooling will result in increasing the relative solid fat content of the droplets. So by modifying the thermal treatment of the cream, it is possible to regulate the size of the crystals in the fat globules and in this way influence both the magnitude and the nature of the fat

Table 11.2. Common Fatty Acids in Cow's Milk Fat (by Mass Fraction)

Fatty Acid	Mass Fraction (%)
Saturated fatty acids	
Palmitic acid	31
Myristic acid	12
Stearic acid	11
Lower (<14 carbon atoms)	11
Unsaturated fatty acids	
Oleic acid	24
Palmitoleic	4
Linoleic	3
Linolenic	1

structuring processes during both churning and subsequent kneading stage, as a means of controlling the textural properties of the butter.

The Dairy Science and Technology Group at the University of Guelph—experts in the formulation and processing of dairy food systems—recommend the following thermal treatments prior to churning and working according to milk fat composition.

Treatment of Hard Fat

In the case where the milk fat contains a high concentration of saturated fatty acids, the following thermal treatment of the cream is recommended as a means of improving the softness of the resulting butter:

- Rapid cooling to about 8°C and storage for about 2 hours at this temperature;
- Reheating gently to 20–21°C and storage at this temperature for at least 2 hours (water at 27–29°C is used for heating);
- Cooling to about 16°C.

Initial cooling to about 8°C induces more rapid nucleation and the formation of a large number of small crystals which in turn incorporate a high proportion of liquid oil. When the cream is gently heated to 20–21°C, partial melting of these crystals takes place, increasing the liquid oil fraction within emulsion droplets. After 1–2 hours most of the high melting point fats will have slowly re-crystallized, without incorporating too much of the low-melting point and liquid oil fractions. Reducing the temperature back to 16°C at this stage causes some additional crystallization of lower melting point fractions resulting in the growth of larger crystals, but with minimal entrapment of liquid oil within the fat crystal structure. By maximizing the relative amount of noncrystallized oil in the emulsion, a softer butter can be produced during the working step.

Treatment of Medium-Hard Fat

- Rapid cooling to about 8°C and storage for about 2 hours at this temperature;
- Reheating gently to 16°C and storage at this temperature for at least 2 hours.

With an increase in the concentration of unsaturated fatty acids, the reheating temperature is reduced from 20–21°C to as low as 16°C. As with the hard fat treatment, there is therefore an initial rapid nucleation and crystallization step which serves to trap a high proportion of the liquid oil. Since the temper-

ing step is now at a lower temperature than that of the hard fat treatment, there is less melting out of these crystals, and so a higher proportion of liquid oil remains entrapped within the crystalline phase. This consequently leads to a firmer product during working.

Treatment of very Soft Fat

For cream containing a high concentration of unsaturated fatty acids the “summer method” of treatment is used. After pasteurization the cream is initially cooled to 20°C. After this stage the cream is cooled to between 6 and 8°C depending on the degree of unsaturation. This crystallization process serves to entrap higher amounts of liquid oil within the fat crystals. In this way the firmness of the butter can be increased.

WORKING

As discussed, the process of churning serves to agglomerate emulsion droplets from cream into butter granules, visible aggregates of fat. After churning, the buttermilk is drained from the fat granules. Salt is added (1–3%) and the granules are then worked. During working, fat moves from globular to free fat, completing the inversion process from initial oil-in-water cream emulsion to the final water-in-oil butter emulsion. During this process, the water droplets decrease in size becoming small enough so as not to be visible in properly worked butter.

During the formation of the water-in-oil emulsion, the water droplets themselves are stabilized through the interfacial coating (Pickering stabilization) of water droplets by fat crystals from the continuous phase which helps to prevent coalescence. Stability of the water-in-oil emulsion is further enhanced through kinetic trapping of droplets by fat crystallization which immobilizes the water droplets within the fat structures. Overworked butter will be too brittle or greasy depending on whether the fat is hard or soft. Some additional water may be added to standardize the moisture content.

Precise control of composition is essential for maximum yield. The final microstructure of butter is given in Figure 11.6. In the case of blended emulsions, where higher concentrations of liquid/unsaturated oils may be added, it is essential that there remains sufficient crystalline fat to maintain droplet stability during working. If solid fat content is dropped too low, the stability of the butter microstructure may be compromised. In such cases,

Butter structure

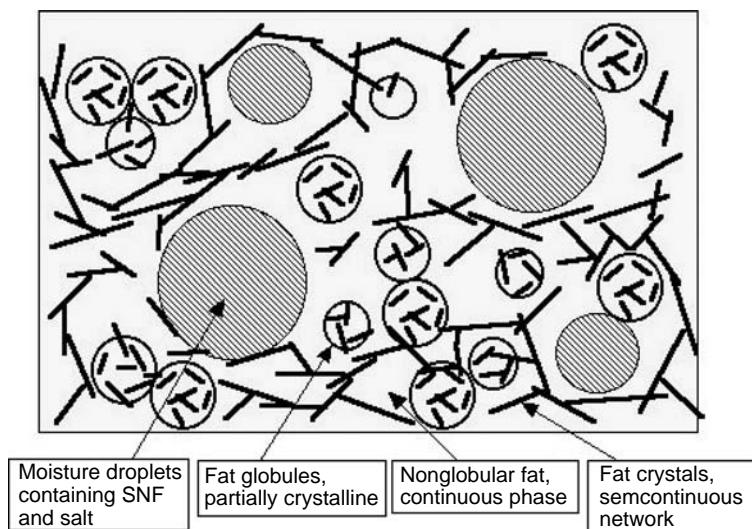


Figure 11.6. Schematic representation of the microstructure of butter.

the stability of the emulsion can be enhanced by the addition of emulsifier (saturated and unsaturated monoglycerides) to the oil phase, which provides a protective coating around the water droplets during working. The use of these is dependent on legislation and regulatory status, and it should be noted that their use may preclude a product from being defined as butter.

EATING

The particular microstructure of butter serves not only to impart long-term physical and microbiological stability on the product, but is also responsible for delivering the appropriate sensory behavior. The particular sensory experience associated with eating butter, or for that matter any other fat continuous spread, is due to the changes in butter microstructure during consumption.

The effects of in-mouth temperature cause a melting of the fat network. Coupled with salivary dilution and oral shear, the semisolid water-in-oil emulsion structure is broken, partially inverting to a coarse oil-in-water emulsion that gives a creamy, rather than a greasy, in-mouth perception (as would be the case if butter consisted of 100% milk fat, or alternatively if the water-in-oil emulsion did not break). In addition, the breaking of the water-in-oil emulsion serves to release salt and milk proteins from the water droplets,

which provides the appropriate delivery of salty taste and dairy flavor notes during eating.

BUTTERMAKING TECHNOLOGY

In recent years, much of the development in butter-making technology has improved the efficiency of the equipment to reduce butterfat losses, increase butter-milk drainage, increase butter yield, reduce power consumption, and ultimately improve profitability.

CREAM

Raw milk for cream and butter manufacture should be of good microbiological quality; for example, an average total bacterial count for raw milk in the United Kingdom might be of the order of <20,000 colony-forming units (CFU)/mL of milk (maximum acceptable is 100,000 CFU/mL). Cream of the required fat content for the process and product is prepared by separating liquid whole milk into skim milk (0.05% fat) and cream (e.g., 38–42% for most continuous buttermakers) in a centrifugal separator. Many plants operate a cold separation procedure (<10°C) to reduce free fat loss into the skim milk although a higher separation efficiency is possible as temperatures increase. Cold-separated cream contains higher quantities of phospholipids which improves whipping properties.

The cream is then pasteurized in a continuous high temperature short time (HTST) plate heat

exchanger normally to a higher temperature than milk pasteurization, before cooling to ageing temperature. The minimum pasteurization temperature/time combination recommended is 72–77°C for 15 seconds for most countries but higher temperatures up to 95°C are frequently used. Flavor taints from animal feed can be removed at the cream pasteurization stage by carrying out the heat treatment under vacuum (vacreation) before cooling the cream. Vacreation, however, can increase fat loss into the buttermilk.

Cooling and ageing of cream, its effect on fat crystallization, and how this part of the process can be modified to improve butter spreading characteristics have been discussed earlier. The cooled cream may be held in cream ageing tanks or, for larger operations, in silos. Slow-moving agitators or intermittent mixing is necessary to prevent separation but care should be taken to avoid damage to the cream. Ripened cream may be produced at this stage for cultured butter. Usually, such cream is pasteurized at a higher temperature than cream for sweet cream butter, cooled to ripening temperature (20–27°C) and inoculated with starter culture (1–2%). Normally, a mixed culture of lactic microorganisms such as *Lactococcus lactis* subsp. *cremoris* (formerly *Streptococcus cremoris*), *Lactococcus lactis* subsp. *lactis* (*Streptococcus lactis*), *Lactococcus lactis* biovar. *diacetylactis* (*Streptococcus diacetylactis*) is added to the cream to insure acid (pH 5.3–4.7) and flavor (especially diacetyl) development. The primary aroma producers are *Lactococcus lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*. Cooling the cream and holding it for several hours controls the extent of the fermentation process and strength of flavor development as well as allowing crystallization of the fat in the cream to be completed before churning. The ripened cream may then be batch churned (see Fig. 11.7).

An alternative process was developed in the mid-seventies by the Netherlands Dairy Research Institute, the NIZO method, which involves adding a mixture of cultured whey concentrate and bacterial culture to sweet cream butter during working. The concentrates, which can vary in composition, add lactic acid, aroma, and flavor compounds to butter, so avoiding the production of lactic buttermilk. There are several advantages to using concentrates to produce ripened or cultured butter rather than ripened cream: (i) the components to be added to the butter can now be stored and prepared without necessitating the production of starter cultures in a factory laboratory, and (ii) sweet cream buttermilk is a more



Figure 11.7. Batch butter churn (APV Unit Systems, Denmark).

valuable waste product and one easier and cheaper to dispose of than lactic buttermilk.

BUTTER FORMATION

Butter is now commonly manufactured using continuous buttermaking machines. These machines have the advantage over the older batch churns (see Fig. 11.7) in terms of consistent production of a butter of uniform quality with low air content (better texture and less oxidation) and improved moisture distribution and smaller water droplet size (improved shelf life and bacteriological quality). A continuous butter-making machine from APV Unit Systems, Denmark, is shown in Figure 11.8; the capacity of such equipment ranges from 500 kg/h to 12,000 kg/h and their flexibility enables sweet, cultured, or whey creams with a range of fat contents to be churned as well as dairy blends of cream mixed with vegetable oil.

Greater operating detail may be seen in the schematic outline of the machine in Figure 11.9. The machine is divided into three sections:

- Churning section
- Separating section
- Working section(s)

Churning Section

The churning section consists of a horizontal cylinder and a multi-bladed beater that sits only a few millimeters from the cylinder wall. Tempered (aged) cream at the desired churn temperature is pumped

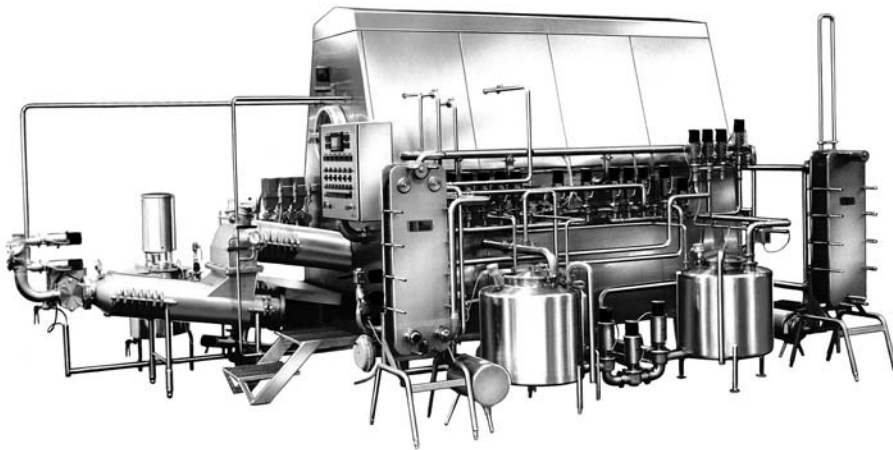
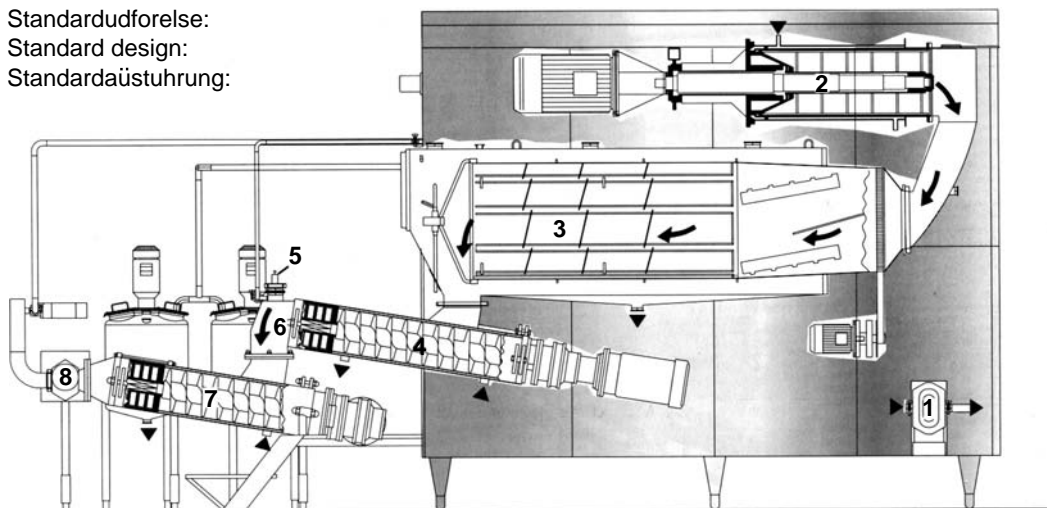


Figure 11.8. Continuous buttermaking machine (APV Unit Systems, Denmark).

Standardudforelse:
Standard design:
Standardaüstuhung:



1. Flødepumpe
2. Kærneafdeling
3. Separeringsafdeling
4. Ælteafdeling I
5. Reguleringsspiæld
6. Vakuunkammer
7. Ælteafdeling II
8. Smørpumpe

1. Cream pump
2. Churning section
3. Separating section
4. Working section I
5. Regulating gate
6. Vacuum chamber
7. Working section II
8. Butter pump

1. Rahmpumpe
2. Butterungsabteilung
3. Trennabteilung
4. Knetabteilung I
5. Reglerplatte
6. Vakuunkammer
7. Knetabteilung II
8. Butterpumpe

Figure 11.9. Schematic outline of a continuous buttermaking machine (APV Unit Systems, Denmark).

into the rear end of the churning cylinder where the beater, operating at speeds of approximately 1,000–1,500 rpm, introduces air into the cream, damages the fat globule membrane, and causes the globules to agglomerate. Selection of beater speed and speed constancy is important to achieve lowest fat loss into the buttermilk and highest moisture content of the buttermilk. The size and moisture content of the butter grains as well as the fat content of the buttermilk are largely determined by the speed of the beater. The churning process only takes a few seconds and a mixture of butter grains and buttermilk then flows into the separating chamber.

Separating Section

This section consists of a horizontal rotating cylinder where two operations are performed. The first part of this section contains beaters to continue churning the mixture of buttermilk and butter grains causing larger clumps to form and more buttermilk to be expelled. A perforated filter, or separation drum, then separates the buttermilk from the butter, while the rotation of the drum encourages further clumping of the grains. Operating parameters of the churning and separating sections such as temperature and churning/rotating speed will be controlled to meet cream and product requirements.

Working Section(s)

Typically in buttermaking machines, there are two working sections linked by a vacuum chamber. Working section 1 comprises both augers for transportation of the butter and also working elements, that is, working vanes and perforated plates. In this section, the butter mass is kneaded, expelling buttermilk before addition of water and/or salt slurry. If the augers operate at too low a speed they will not squeeze sufficient buttermilk out of the butter.

Butter is forced through a regulating gate between working sections 1 and 2. By adjusting the apertures of the gate, the counter pressure on the butter can be adjusted and hence the amount of buttermilk expelled can be regulated. Passing through the apertures of the regulating gate greatly increases the surface area of the butter but by applying a vacuum in the chamber connecting the two working sections, it is possible to reduce the air content in the butter from 5 to 6% to less than 0.5%. Deaeration improves shelf life and appearance of the butter, resulting in a more closely textured product than is found in traditionally worked butter.

Working section 2, like the first working section, consists of augers and working elements, however the auger speed is usually two or three times higher than in working section 1. The function of the second working section is to carry out the final working of the butter and insure water and salt are evenly distributed throughout the butter, with water droplet size as small as possible, approximately 5 μm , to prevent undesirable microbial growth during storage. Overworking, however, will produce a sticky butter which is difficult to pack. Salt is introduced into butter in modern equipment as a slurry via a computer-controlled pump. The slurry is a mixture of ultrafine salt grains ($<20 \mu\text{m}$) and water in a 50:50 ratio. Traditional batch churns had a working time of 30 minutes which was ample to allow salt to dissolve in the aqueous phase of the butter. Modern continuous buttermaking equipment has a working time of approximately 5 minutes which would be insufficient for dissolution of salt, hence the use of pumped salt slurries.

Packing

Butter may be packed in bulk in 25-kg polythene-lined cardboard cartons for chill or frozen (-18 or -25°) storage, printed as 250 g or 500 g retail blocks, and wrapped in parchment or lined foil, or extruded into attractive plastic tubs similar to those used for margarines and other spreadable fats. Re-packing bulk butters for retail purposes usually requires reworking of the butters first. The temperature of the bulk butter is raised to 5 – 8°C either by holding it in a store at this temperature or by using microwave tunnel heaters. It is then worked to blend and standardize the salt and moisture content of the final product. An alternative process involves chopping the frozen butter into thin strips while maintaining the butter temperature at 0 – 2°C . The butter is then worked in the blender section several times and deaerated before being packaged in retail units.

LOW-FAT BUTTER

The traditional process for producing low-fat or half-fat butter (40% fat) is based on using butter oil into which an aqueous phase such as milk or buttermilk, and stabilizers, emulsifiers, colorings, flavors, and antioxidants are blended. This mixture is then chilled and crystallized in a scraped surface heat exchanger. An alternative process (APV patented method) may be employed that uses standard butter directly (see Fig. 11.10). In this process, the butter is worked and

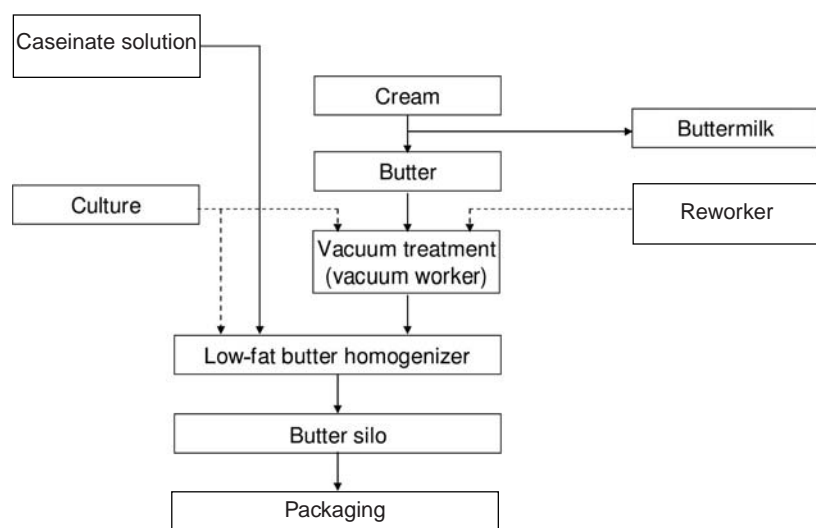


Figure 11.10. Low-fat butter production (APV Systems, Denmark).

de-aerated in a vacuum working section, where it is also heated gently before being pumped into a butter homogenizer. The softened butter is first dosed with a pasteurized solution of sodium caseinate before the mixture is homogenized to insure that a homogeneous blend of the butter and protein solution is achieved, with small (ca. 5 μm) well dispersed water droplets. The degree of homogenization depends upon the milk fat composition of the original butter, homogenization temperature, and the required fat content of the finished product. Starter culture and salt can also be added at this stage. Following homogenization the blend is pumped into a silo and from there to a scraped surface heat exchanger for cooling before packing.

SPREADABLE BUTTER

Butter is recognized as a high-quality natural product with a unique flavor; unfortunately, it is also well known that butter is hard and virtually unspreadable at refrigerator temperature and this property makes it compare unfavorably with dairy spreads and table (tub) margarines. The primary reason for the firmness of butter is the high content of saturated fatty acids in milk fat that are solid at low temperatures (see Table 11.2). Legislation prohibits any fat other than milk fat to be present in butter and this limits options available to butter manufacturers to improve butter spreadability. As mentioned earlier, physical working to disrupt the three-dimensional butter structure or application

of a cream tempering regime to alter fat crystal number and size can achieve moderate success. However, greater success has been achieved in recent years by modifying the fatty acid composition of the milk fat through changes to the dairy cow's diet.

There are many reports in the literature concerning modification of the cow's diet to increase the content of unsaturated fatty acids in milk fat, thus reducing solid fat content. These have been reviewed by Ashes et al. (1997) and more recently by Murphy (2000). As the long chain fatty acids in milk fat, that is, all the C18 acids and approximately 50% of C16 acids, originate from the cow's diet, they have been the focus for dietary manipulation of milk fat. In a cow's digestive system (rumen), microorganisms normally hydrolyze and hydrogenate dietary lipid, resulting in mainly saturated and partially saturated fatty acids entering the blood stream to be transported to the mammary gland where milk is biosynthesized. In the mammary gland the delta-9 stearoyl desaturase enzyme then converts a significant proportion of the saturated C18:0 fatty acid, stearic acid, into the monounsaturated C18:1 fatty acid, oleic acid.

"Pure" naturally spreadable creamery butter was first launched under the Dromona label by Dale Farm Limited in Northern Ireland in 1999 (launched under "Pure" brand name in 2003), and is one of the few dietary modified spreadable butters that has made it onto the commercial market. Milk producers were recruited onto a scheme to feed their cows a special concentrate containing whole rapeseed during

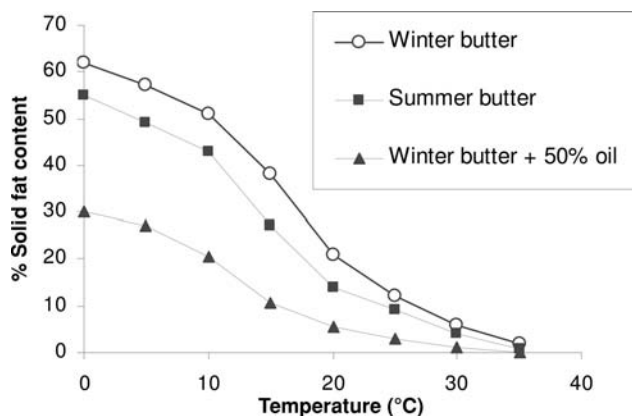


Figure 11.11. Solid fat content versus temperature curves for winter butter, summer butter, and a blend of winter butter and 50% vegetable oil (measured by nuclear magnetic resonance). (Adapted from Fearon, 1986).

the summer months when the animals were grazing fresh grass. Inclusion of the high-oil supplement in the form of an intact oilseed helped protect the unsaturated fatty acids in the rapeseed from the hydrogenating activity of the microorganisms while also insuring normal animal digestion in the rumen. The combination of partially protected rapeseed and fresh grass resulted in the production of milk containing a high content of unsaturated fatty acids, in particular the monounsaturated fatty acid C18:1 oleic acid, with a milk fat iodine value of <45 g iodine/g fat (Fearon et al., 2004). This resulted in sufficient softening of the butter at low temperatures to markedly improve spreadability while maintaining product body at room temperature.

Previous spreadable butters have been produced by feeding large amounts of rumen-protected polyunsaturated oils to dairy cows which substantially increased the content of polyunsaturated fatty acids in the milk by by-passing rumen hydrogenation. However, rapid oxidation of the raw milk and butter was a major problem (Banks and Christie, 1990). As the main unsaturated fatty acid in “Pure” butter is a monounsaturated acid (oleic acid), oxidation was not a problem (Fearon et al., 2004). The product commands a premium price while milk producers also receive an increased payment to compensate for higher feed costs and reward them as members of the scheme.

An alternative approach to produce spreadable butter within “butter” labeling regulations has been to incorporate a lower-melting fraction of milk fat, or a blend of milk fat fractions, into butter or cream. Solid fat content in milk fat from pasture-fed dairy cows may be as high as 50% at refrigerator temper-

atures while butter produced under winter feeding conditions (conserved forage and concentrates) will be higher still (see Fig. 11.11). In order to achieve optimum spreadability in a spreadable fat product at 5°C, it would be necessary to reduce the solid fat content to 30–40%, but this must be balanced by a solid fat content of 10–20% at room temperature (20°C) to support product body and prevent oiling off. Blends of fractions with different melting points would seem to be preferred over addition of a single low-melting fraction to achieve these properties in the final butter (Kaylegian and Lindsay, 1992).

NUTRITIONALLY ENHANCED BUTTER

Modification of milk fat composition through the cow’s diet has also been employed to improve the nutritional properties of butter. It is widely recognized that the dietary intake of ω -3 PUFA in many populations is substantially lower than their ω -6 PUFA intake, thus greatly increasing their risk of serious inflammatory diseases such as cardiovascular disease (Simopoulos, 2003). Dietary guidelines around the world for fat consumption recommend certain intakes of ω -3 and ω -6 PUFA to remedy the problem (Lunn and Theobald, 2006; see Table 11.3). Raising the content of ω -3 PUFA in basic food commodities that are consumed in relatively large amounts may present an opportunity to redress the balance. The content of alpha-linoleic acid (ALA, ω -3 PUFA) in cow’s milk fat may be increased substantially by feeding dairy cows rumen-protected lipid supplements containing ALA, for example, protected flaxseed (Goodridge et al., 2001). However, it is more difficult to increase the content of longer chain ω -3 PUFA, EPA, and

Table 11.3. Dietary Guidelines for Daily Fat Consumption (% of Energy) and Recommended Fatty Acid Intake (Lunn and Theobald, 2006)

Region	Fat	ω -3 PUFA	ω -6 PUFA
USA and Canada	20–35	0.6–1.2	5–10
Europe	<30	200 mg DHA/EPA; 2 g ALA	4–8
FAO/WHO	35	LA:ALA = 5–10:1	4–10
UK	<35	>0.2 (450 mg DHA/EPA)	>1

ω -3 PUFA: DHA, docosahexaenoic acid C22:6; EPA, eicosapentaenoic acid C20:5; ALA, alpha-linolenic acid C18:3.

ω -6 PUFA: LA, linoleic acid C18:2.

DHA, in milk fat because of poor uptake of these acids at the cow's mammary gland (Lock and Bauman, 2004).

Conjugated linoleic acid (CLA) has been of interest to health and medical professionals because of its bioactive properties including possible effects on body composition, antidiabetic effects, antiatherogenic effects, and inhibition of carcinogenesis (Tricon et al., 2005). CLA is the term applied to isomers of octadecadienoic acid (C18:2) containing conjugated double bonds. Products obtained from ruminants such as meat, milk, and dairy products are the main source of CLA in the human diet. Up to 90% of CLA in milk fat is the *cis*-9, *trans*-11 isomer which arises naturally from biohydrogenation of dietary linoleic and linolenic acid directly, or via desaturation of the CLA precursor, vaccenic acid (*trans*-11 C18:1). The concentration of CLA in milk normally ranges between ca. 0.5 and 1.7 g/100 g total fatty acids, varying with season and associated animal feeding regimen. Research has focused on increasing the content of CLA in milk and dairy products through feeding unsaturated lipid supplements high in linoleic and linolenic acids to dairy cows, with the added bonus that such feeding strategies also increase MUFA and reduce the content of saturated fatty acids in the milk fat (Lock and Bauman, 2004). CLA-enriched milk has been used to manufacture butter and cheese and although both products were less firm than the nonenriched control products, flavor, and storage properties were acceptable and similar to the control products (Jones et al., 2005).

DAIRY SPREADS

It is apparent that to make a spreadable milk fat-based product that can compete with spreadable margarine products, the proportion of unsaturated fatty acids must be substantially increased. This can be achieved outside of the butter regulations by adding liquid

vegetable oil to the butter or cream to reduce solid fat content. This is the basis of dairy spreads and there are now a number of different types of dairy spreads available with improved spreading properties and a range of fat contents (see Table 11.1).

The traditional dairy blend such as the Swedish product "Bregott," launched in 1976, was prepared by injecting vegetable oil, usually canola or rapeseed, into the cream prior to churning in a continuous buttermaker. Since the blend of butter and oil is softer and more spreadable than butter at refrigeration temperatures, it is necessary to churn the cream and oil mixture at a lower temperature (5°C), and maintain this temperature throughout the process. Obviously, it is important that the vegetable oil selected does not solidify at this temperature. The final product typically has a fat content similar to butter, but with 15–25% of the milk fat being replaced by vegetable oil. Alternatively, injection of the vegetable oil into the butter rather than the cream has the advantage of reducing vegetable oil loss into the buttermilk and the high-energy cost/low-temperature conditions for churning cream/vegetable oil blends are not required.

The concentration of vegetable oil in these traditional dairy spreads is limited; this is because as butter is heated, it displays a melting curve of solid fat content versus temperature that is sigmoidal in shape, with a steep reduction in solid fat between 10 and 20°C. The addition of high amounts of vegetable oil results in a product with unacceptably low solid fat content, showing poor body and "oiling off" at room temperature (20°C; see Fig. 11.11). However, by adopting some of the technology and formulation aspects from the margarine industry, dairy spreads with higher proportions of vegetable oil and lower solid fat contents can be produced.

An important factor is the use of scraped surface heat exchangers which can produce butter-like products from high oil formulations in the absence of air. A scraped surface heat exchanger is typically a

tubular heat exchanger cooled by a liquid refrigerant (e.g., ammonia) with scraper blades mounted on a central shaft which rotates continuously, removing crystallized fat from the inner tube surface to promote rapid cooling and crystal nucleation in a short residence time (seconds). Usually, scraped surface heat exchanger units are employed in combination with crystallizer units that hold a larger volume and have a longer residence time (several minutes). These units work the product between a series of metal pins fixed within the crystallizer tube and others mounted on a rotating central shaft, shearing the product, preventing formation of large crystal networks, and dispersing moisture droplets. The majority of crystallization of the fat takes place in the crystallizer unit(s) and the blend may then be transferred to resting tubes to continue crystallization, although with less shear, before packing.

Most manufacturers of spreads produce a range of products of differing fat contents and compositions to maximize their market. For example, Dairy Crest, a major British manufacturer of butter and spreads, includes products within their portfolio of spreadable fat products with fat contents that range from 72% with a significant proportion of milk fat present ("Clover") to as low as 19% ("St Ivel Gold" range) and containing dairy ingredients but no milk fat. "Clover" (72% fat) is a mixed fat product that is produced using churn technology. It is composed of vegetable oils, buttermilk (29%), water, skimmed milk, cream, salt, emulsifier, flavorings, vitamins A and D, and color. Approximately, 50% of milk fat in "Clover" is replaced by vegetable oil and this necessitates the inclusion of some form of hard fat to provide a solid base that improves product body and reduces oiling off at room temperature. "Clover" has a slightly higher moisture content ($\leq 20\%$) than butter ($\leq 16\%$), and to aid dispersal of the moisture an emulsifier is added to the vegetable oil/buttermilk mixture.

In contrast, "St Ivel Gold Extra Light + Omega-3" (19% fat) is an example of the new generation of very low-fat spreads designed to appeal to health-conscious consumers. Its low-fat content of just 19% fat will attract calorie-aware consumers, yet by enriching the spread with long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) C20:5, and docosahexaenoic acid (DHA) C22:6; it may also play an important role to help consumers increase their daily intake of these essential fatty acids, as recommended by the Government and other bodies. The aqueous phase in low-fat spreads is very important. In the St Ivel product, the aqueous phase is based on

skim milk, buttermilk, and modified starch and it is likely that interactions between the starch and protein ingredients in the blend are responsible for emulsion stability and viscosity. Other products have employed blends of maltodextrin and gelatine to similar effect. The amount and nature of structuring agent in the aqueous phase is critical, not only because of stability of the emulsion and viscosity but also because of effects on organoleptic properties.

There is little information in the open literature about the manufacture of very low-fat products and what combination of technology and ingredients are needed to achieve desirable texture, stability, and mouthfeel. Both water-in-oil-type and oil-in-water low-fat spreads are available. Low- and very low-fat dairy spreads are competing with the margarine-type range of products rather than with butter or higher fat dairy spreads, hence dairy elements within such products will have more to do with flavor and dairy image and less with the functional fat component. However, inclusion of dairy emulsifier and flavorings such as milk protein introduce their own problems in the manufacturing process of spreads. Milk proteins favor oil-in-water emulsions, encourage formation of larger water droplets and reduce the stability of water-in-oil products under such conditions as are applied during the latter stages of processing and tub filling (Moran, 1994).

Following the introduction of worldwide regulations requiring the labeling of the *trans* fatty acid content in foods on health grounds, there is a drive by manufacturers of processed foods to reduce or eliminate industrial *trans* fatty acids from their products. This has led to replacement of the blend of hydrogenated and semi-hydrogenated vegetable oils that helped insure plasticity in many of the spreadable fat products by ingredients containing no, or only trace amounts, of *trans* fatty acids. One option is to blend liquid vegetable oils and butter with natural hard fats in proportions that vary with the fatty acid composition of winter (hard) and summer (soft) milk fat, to insure the final product has sufficient solid fat content for good body at higher temperatures and yet continues to spread easily at refrigerator temperatures. More recently, there has been considerable research into structured lipids to allow production of tailor-made fats with desired nutritional, chemical, and physical properties (Osborn and Akoh, 2002). Generally, structured lipids are triacylglycerols that have been modified to incorporate new fatty acids or to change the position (distribution) of existing fatty acids along the glycerol backbone by chemically or

enzymatically catalyzed reactions or genetic engineering. Inclusion of structured lipids in mixed fat spreads is a novel approach to achieve products with good spreading properties and a relatively high content of milk fat or butter, but having a low trans fatty acid content.

QUALITY ASSURANCE

The International Dairy Federation (IDF), in association with other international standard organizations including the International Standards Organisation (ISO) and the Association of Official Analytical Chemists (AOAC), published a guide to analytical quality assurance and good laboratory practice (IDF, 1993) and an inventory of methods of analysis and sampling for milk and milk products (IDF, 1996). The publications are intended to define the role of quality assurance in dairy laboratories and catalogue a list of adopted methods of sampling and analysis needed to support compositional analysis of milk and dairy products. Methods have been updated as new products and techniques have been developed. Total fat content in spreadable fats, to meet EC Council Regulation 2991/94 labeling requirements, is one such example of method development.

TOTAL FAT

Selection of an internationally recognized reference method to measure total fat was hampered by reports of practical problems and/or poor precision when established worldwide reference methods (e.g., Rose Gottlieb method, Schmid-Bondzynski-Ratzlaff method, and the previous IDF Standard 80 (1977)) were applied to the range of spreadable fats available, from high-fat low-moisture butter to low-fat high-moisture spreads (Envers et al., 1999).

Following collaborative studies and interlaboratory trials, a gravimetric solvent extraction method proposed by Envers et al. (2000), was adopted as a British Standards and European Standards reference method for determination of fat content in butter, edible oil emulsions, and spreadable fats (BS EN ISO 17189:2003). The method is based on petroleum ether as the fat solvent rather than a mixture of petroleum ether and diethyl ether to improve safety and reduce environmental impact. It uses a smaller sample size (1–2 g) and includes centrifugation to achieve more rapid phase separation and reduce the number of extractions required for full recovery of the fat. The reference method also includes two

modification proposed by Envers et al. (2000) as practical measures to improve extraction recovery and repeatability: (i) A small amount of ethanol is added to the petroleum ether phase to reduce cloudiness that cannot be overcome by the addition of salt (sodium or calcium chloride) or extended centrifugation times, and (ii) Congo-red solution is added to color the serum phase and reduce the risk of transferring some of the serum phase with the solvent phase during separation should transparent serum phases arise.

SPREADABILITY

Measurement of butter consistency is important as this property affects consumer acceptability of the product. Wright et al. (2001) reviewed the rheological properties of butter during and after manufacture, and the methods available to measure these. Much of the research to relate instrumental measures of butter spreading characteristics to consumer perception of spreadability was carried out in the 1960s–1980s and there have been changes since then in milk production practices, milk fat composition, butter manufacture, butter storage and handling and even the nature of competing products (influence consumer expectations). Consumer perception of butter spreading properties encompasses an evaluation of both the non-Newtonian plastic properties of butter as well as its viscoelastic flow behavior. Nevertheless, it is widely accepted that there is a close correlation between butter hardness and proportion of solid fat present and that these in turn are inversely related to butter spreadability. This means that an instrumental assessment of butter hardness (e.g., by cone penetrometry or sectility testing), although limited, can still provide a useful indication of butter spreadability.

ADULTERATION OF MILK FAT

There are traditional chemical tests available to confirm the authenticity of milk fat (Reichert-Meißl, Polenske, and Kirschner values) based on the content of water-soluble and water-insoluble fatty acids present (Fearon, 2003). Other more modern tests include use of gas chromatographic (Ulberth, 1994) or near infra-red (Sato et al., 1990) analysis to provide information on fatty acid composition of the fat, which is then used to calculate ratios of certain acids. Production of milk with tailored milk fat composition to improve the functional (nutritional and physical)

properties of dairy products, may however challenge the basis of these tests.

MICROBIOLOGICAL QUALITY

The microbiological quality of any dairy product is also of importance and will obviously be affected by the quality of the original milk. As mentioned previously, cream for butter manufacture is normally pasteurized at a minimum of 72–77°C for 15 seconds although there is a trend for flash heating at higher temperatures, 88–93°C, to be employed to give a nutty, slightly caramelized flavor to the butter. However, oxidative stability of the cream decreases as temperatures increase above 60°C because copper, a metal pro-oxidant, can migrate from the serum into the fat globules. Microbiological guidelines for acceptance of unsalted butter into intervention storage are typical of standards applied across the world and require coliform counts in butter to be <1 CFU/g. It is also standard that dairy products should be absent from pathogens such as *Listeria monocytogenes* and *Salmonella*.

The appearance, consistency, and flavor/aroma of butter are usually defined as quality parameters and butter grading involves sensory evaluation of a core of butter which is then scored for these attributes. Good quality sweet cream butter should have a smooth, close-textured body with a “dry” even-colored appearance, no visible salt, and a clean butter aroma. Common defects that reduce butter quality include:

- *Appearance*: streaky color, mottling, undissolved salt, free moisture, mould present;
- *Consistency*: brittle, crumbly, sticky, greasy;
- *Flavor and aroma*: unclean, acid, musty, feed flavor, over-salted.

A full description of butter grading standards and descriptors is published by the United States Department of Agriculture (1989). A list of additives permitted in butter, that is, coloring agents such as annatto extracts and carotenes, and acidity regulators, is specified in the Codex Standard for Butter (1999).

In the manufacture of other spreadable fats, the characteristics of the emulsion formed, determine its quality and shelf life (Charteris, 1985). The small size of the water droplets within the emulsion (1–20 µm) limits the ability of microorganisms to grow and move between droplets. Hence, it is important to control formation of water droplets and avoid their coalescence and channel formation during manufacture. This aspect of control is even more critical in

the manufacture of reduced and low-fat products with higher moisture content. Mould spoilage of spreadable fats has been occasionally reported, and while not a safety hazard, they can cause off-flavors in the product. The addition of salt and preservatives such as sorbate reduces spoilage problems while even ingredients such as pH correcting agents, emulsifiers stabilizers, antioxidants, can play a role in inhibiting microbial growth. These ingredients may also be a source of contamination so microbiological quality specification should include ingredients (Delamarre and Batt, 1999).

CONCLUSION

Interest by consumers in diet and health seems to drive butter production toward a niche market that appreciates the high quality, unique flavor, and natural image of butter. Butter production is likely to follow a similar trend as with other products within the dairy industry (liquid milk, cheese, yogurt, ice cream), with improvements in functional properties, both physical and nutritional. If maintaining a “natural” image for butter is critical, it would follow that such changes would be achieved through the dairy cow, for example, by diet or genetics. Naturally spreadable butters have been mentioned previously but “healthier” butters enhanced with omega-3 PUFA or enriched with CLA (for many potential and varied health benefits) have also been achieved through modification of the cow’s diet. A growing sector within the butter industry is also the production of butter-based ingredients for the food manufacturing industry such as ready-mixed butter-based mixtures of butter, flour, sugar, or vegetable oil. Butter will continue as a premium product but development of nutritionally enriched high-milk fat dairy spreads with improved omega-6:omega-3 ratios will provide stern competition.

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12

Cheese

Tanoj K. Singh and Keith R. Cadwallader

Introduction
Manufacture of Cheese
Biochemical Reactions During the Manufacture and Ripening of Cheese
 Glycolysis
 Lipolysis
Characterization of Key Aroma Impact Compounds of Cheeses
 Aroma Impact Compounds of Feta and Pasta Filata Cheeses
 Aroma Impact Compounds of Goat Milk Cheese
Specific Flavors or Off-Flavors in Cheeses
Taste Compounds in Cheese
Conclusions
References

INTRODUCTION

Cheese is the generic name for a group of fermented milk-based food products. A list of 1,400 cheese varieties is maintained on website www.cdr.wisc.edu (Jim Path, University of Wisconsin). Cheesemaking originated as a crude form of food preservation. The preservation of cheese is as a result of the combined action of:

- *Dehydration*—Cheese is a medium-moisture food, containing about 30–50% moisture. The stability of foods is inversely related to moisture content. The water activity (a_w) of cheese varies from 0.98 to 0.87 and these values are highly correlated with the total nitrogen and ash content (mainly NaCl). Biochemical reactions that occur during the ripening of cheese contribute to the depression of a_w by increasing the number of dissolved low molecular mass compounds and ions.

- *Acid*—During the manufacture and ripening of cheese, starter bacteria ferment lactose to lactic acid. The pH of Cheddar cheese is about 5.0–5.2.
- *Antibiotic* production by starter bacteria.
- *Anaerobic condition*.
- *Addition of NaCl*.

In the past, the stability of cheese was the primary consideration but is no longer the principal objective; today, it is more important to manufacture cheese of consistently good quality, flavor, and texture, that is, balanced ripening. Flavor development in cheese is a complex and slow process involving chemical and biochemical conversions of milk constituents. Lactic acid bacteria (LAB) form the main microbial flora in the cheeses and are essential for the biochemical conversions that determine the specific flavor profiles. However, additional cultures such as propionic acid bacteria in Swiss-type cheeses and various aerobic cultures, for example, *Brevibacterium*, *Arthrobacter*, *Staphylococcus*, *Penicillium*, and *Debaryomyces*, for the surface-ripened cheeses (Engels et al., 2003). In addition to starter organisms, native milk enzymes, coagulant, enzyme(s) added as ripening aid and NSLAB can also play an important role.

In the first section of this chapter, the general steps involved in the manufacture of cheese are considered. The second section deals with the literature concerning the biochemical reactions that occur during the manufacture and ripening of cheese. The third section of this chapter includes a discussion on the flavor chemistry of various types of cheeses. Next section deals with chemistry involved in the generation of specific off-flavors in cheeses. The last section of this



Figure 12.1. General description of steps involved in the cheese manufacture. (Modified from Singh et al., 2003a.)

chapter presents an overview of the taste compounds in the cheeses.

MANUFACTURE OF CHEESE

Although some soft cheese varieties are consumed fresh, that is, without a ripening period, the vast majority of rennet-coagulated cheeses are ripened for 4 weeks to 2 or more years, during which the characteristic flavor and texture of the individual varieties develop. The manufacture of rennet-coagulated cheeses can be divided into two more or less distinct phases: (1) conversion of milk to curd, which is essentially complete within 24 hours; and (2) ripening of the curd (Fig. 12.1).

Cheese manufacture is essentially a dehydration process in which the fat and casein in milk are concentrated between 6- and 12-fold, depending on the variety. The manufacturing phase might be defined as those operations performed during the first 24 hours, although some of these operations, for example, salting and dehydration, may continue over a longer period. Although manufacturing protocols for individual varieties differ in detail, the basic steps listed above are common to most varieties.

Cheese manufacture commences with the selection of milk of high microbiological and chemical quality. Most cheese milk is now pasteurized just before use but raw milk is still used in both commercial and farmhouse cheese making. In general, cheese made from raw milk develops the characteristic Cheddar flavor more rapidly, reaching its best flavor at 3–6 months (Price and Call, 1969). Cheese made from pasteurized milk takes twice as long as that made from raw milk to develop the same flavor intensity and ripens more slowly than raw milk cheese (Fox, 1993). McSweeney et al. (1993) compared the quality

of Cheddar made from raw, pasteurized, or microfiltered milks. The cheeses from pasteurized or microfiltered milk were of good and equal quality; although raw milk cheese was down-graded because of its intense atypical flavor, which developed much faster than that of the other cheeses. Peptide pattern on urea-polyacrylamide gel electrophoretogram of the three cheeses were indistinguishable throughout ripening, but the rate of formation of water-soluble nitrogen was faster in the raw milk cheese. The number of lactobacilli was about 10-fold higher in the raw milk cheese than in the other two and the species of lactobacilli also differed. It was concluded from the above results that lactobacilli were responsible for differences in proteolysis in the cheese made from raw milk, particularly with regards to the formation of short peptides and free amino acids. The species of lactobacilli involved in faster ripening and development of flavor intensity in raw milk cheeses appear to have been killed by pasteurization. Lynch et al. (1994) made aseptic cheeses with selected species of lactobacilli added as adjunct starters. Experimental cheeses showed slightly higher levels of free amino acids but no significant differences in flavor intensity and texture were found between control and experimental cheeses. From the above studies, it appears that pasteurization of milk prior to cheese manufacture influences both the extent and characteristics of proteolysis during Cheddar cheese ripening. Pasteurization of milk causes very limited heat-induced interaction of whey proteins with casein and results in the retention of additional whey proteins in cheese beyond the normal amount which is soluble in the aqueous phase of cheese. The presence of heat-denatured whey proteins in cheese may influence the accessibility of caseins to proteinases during ripening (Lau et al., 1991).

Acidification during cheese manufacture is one of the primary events in the manufacture of most, if not all, cheese varieties and involves the fermentation of lactose to lactic acid by selected LAB or, in traditional cheesemaking, by the indigenous microflora. The rate and point of the process at which lactic acid is principally produced is characteristic of the variety; for example, in Cheddar-type cheese, most acid is produced before molding, while in most other varieties, it occurs mainly after molding. Acid production affects almost all facets of cheese manufacture and hence cheese composition and quality. The amount of acid has a marked effect on the level of proteolysis in the resulting cheese. The activity of the coagulant during manufacture and the retention of coagulant depend on the amount of acid produced during the initial stages of manufacture.

The role of pH in cheese texture is particularly important because changes in pH are related directly to chemical changes in the protein network of the cheese curd. As the pH of the cheese curd decreases, there is a concomitant loss of colloidal calcium phosphate from the casein micelles and, below about pH 5.5, a progressive dissociation of the sub-micelles into smaller aggregates (Lawrence et al., 1987). The solubilization of colloidal calcium phosphate, among other factors, affects curd (cheese) texture, stretchability, and meltability.

The manufacture of Cheddar, as with most other ripened cheeses, begins with coagulation of the milk by rennet. It is now well known that rennet coagulation is a two-step process, the first involving the enzymatic formation of para-casein and peptides, the second involving the coagulation of para-casein by Ca^{2+} at temperature $>20^\circ\text{C}$. Both stages, especially the primary phase, are now fairly clearly understood. Chymosin in rennet specifically cleaves κ -casein at Phe¹⁰⁵–Met¹⁰⁶, which leads to the release of the hydrophilic caseinomacropptide (κ -CN f 106–169) part of κ -casein, located at the surface of the casein micelles. When intact, the micelles are kept colloidally dispersed in milk by steric and electrostatic repulsion involving the negatively charged caseinomacropptide part of κ -casein (Dalgleish, 1993). The casein micelles become unstable following the removal of these hydrophilic peptides; then, at an appropriate temperature (e.g., 30°C), the milk coagulates under the influence of Ca^{2+} in the medium (Dalgleish, 1993).

The majority of cheeses are produced by enzymatic (rennet) coagulation. With a few exceptions, such as Serra de Estrela (Portugal) in which a plant

proteinas, from the cardoon flowers of *Cynara cardunculus*, is used. The acid proteinases from *Rhizomucor miehei* and less frequently *R. pusillus* or *Cryphonectria parasitica* are widely used for commercial cheese production in many countries. The introduction of fermentation-derived chymosin has limited the use of these enzymes (Fox et al., 1994).

A rennet milk gel is quite stable if maintained under quiescent conditions but if it is cut or broken, syneresis occurs rapidly, expelling whey (Fox, 1993). During cheesemaking, cutting of curd into small pieces gives faster (initial) syneresis which is proportional to the area of the surface exhibiting syneresis (Walstra et al., 1987). The rate and extent of syneresis are influenced by milk composition, especially Ca^{2+} , casein, pH of the whey, cooking temperature, rate of stirring of the curd–whey mixture, and time. After curd making and drainage, one of the following procedures is usually applied:

- Molding the curd, followed by further drainage under its own weight; this is applied only for soft cheeses.
- Molding and pressing the curd; this is the common method for semi-hard and several hard cheeses.
- Letting the curd rest for a considerable time to develop sufficient acidity (often while allowing the curd to flow: cheddaring) after which the coherent curd mass is cut into fairly small pieces (milling), salted, molded, and pressed.
- Intensively working the already-acidified curd (pH ~ 5.3) at quite a high temperature, as is done in making pasta-filata cheeses.

During several of these processing steps, the curd may lose considerable moisture (Dejmek and Walstra, 2004). In Cheddar-type cheese during cheddaring, the drained mass of curd is allowed to spread laterally for a considerable time. This leads to higher moisture content (1–2% more water) compared to curd kept for the same time but which is prevented from spreading. The main cause of the differences is presumably that the flow of curd promotes deformation of curd grains thus closing pores and hindering drainage of any moisture still leaving the grains due to syneresis. The composition of the finished cheese is to a very large degree determined by the extent of syneresis and since this is readily under the control of the cheese maker, it is here that the differentiation of individual cheese varieties really begins (Fox, 1993).

The last manufacturing operation is salting. Salting may be performed by mixing dry salt with broken

or milled curd at the end of manufacture (e.g., Cheddar); by submerging cheese in brine (e.g., Dutch- and Swiss-type cheeses); rubbing dry salt on the surface of the cheese (e.g., Blue-type cheeses) or by a combination any two of these. Salt exercises one or more of the following functions:

- Direct modification of flavor: unsalted cheese is insipid which is overcome by 0.8% salt
- Promotes curd syneresis and thus regulates the moisture content of cheese
- Reduces a_w
- Influence the activity of rennet, starter and NSLAB and of their enzymes, and indigenous milk enzymes
- Suppresses the growth of undesirable non-starter microorganisms
- In Cheddar-type cheeses, salt, by its influence on postcheddaring starter activity, controls the metabolism of lactose and thus the pH of the fresh cheese, which in turn affects the rate of maturation and cheese quality (Fox, 1987)

Some cheeses are consumed fresh; however, most varieties are not ready for consumption at the end of manufacture but undergo a period of ripening, which can vary from about 3 weeks to more than 2 years, the duration being generally inversely related to the moisture content of the cheese. Cheeses are ripened under controlled temperature conditions (from 3 to 25°C, depending on the variety) and possibly under controlled humidity.

Curds for different cheese varieties are recognizably different at the end of manufacture, mainly as a result of compositional and textural differences arising from differences in milk composition and processing factors. The unique characteristics of the individual cheeses develop during ripening, although in most cases the biochemical changes that occur during ripening, and hence the flavor, aroma and texture of mature cheese, are largely predetermined by the manufacturing process, that is, by composition, especially moisture, salt, and pH, by the type of starter and in many cases by secondary inocula added to, or gaining access to, the cheese milk or curd (Fox, 1993).

BIOCHEMICAL REACTIONS DURING THE MANUFACTURE AND RIPENING OF CHEESE

Considerable knowledge on the principal changes and pathways involved in manufacture of fermented

milk and cheese ripening has been accumulated over the last several decades. The three primary biochemical processes are:

- Glycolysis
- Lipolysis
- Proteolysis

The relative importance of each of these processes depends on the type of fermented dairy product. These primary changes are followed and overlapped by many secondary catabolic changes, including deamination, decarboxylation, and desulfurylation of amino acids, β -oxidation of fatty acids and even purely synthetic chemical changes, for example, esterification. The above-mentioned primary reactions are mainly responsible for the basic textural changes and are also largely responsible for the basic flavor of fermented dairy products. However, secondary transformations are mainly responsible for the fine aspects of cheese flavor and for modification of cheese texture. Glycolysis, lipolysis, proteolysis, and related reactions are further discussed in the next few sections.

GLYCOLYSIS

During cheese manufacture (e.g., Cheddar, Dutch, Camembert, Blue, etc.), mesophilic starter bacteria ferment lactose to (mainly L^+) lactic acid (see Fig. 12.2).

In the case of Cheddar-type cheeses, most of the lactic acid is produced in vat before salting and molding whereas for most other varieties, acidification occurs mainly after the curds have been placed in molds. During manufacture or shortly thereafter, curd pH reaches ~ 5.0 , but the rate is characteristic of a variety (6–24 hours). Even after losing $\sim 98\%$ of the total lactose in milk in the whey as lactose or lactate, cheese curd can still contain as much as 1.5% lactose at the end of manufacture (Huffman and Kristoffersen, 1984). The pH decreases after salting, due to the action of starter, at S/M levels $< 5.0\%$ but at high values of S/M, starter activity decreases abruptly (Fox et al., 1990) and the pH remains high. The grade assigned to the cheese also decreases sharply at S/M levels $> 5.0\%$ (Lawrence and Gilles, 1982).

Commercial lactic cultures are stimulated by low levels of NaCl but are very strongly inhibited $> 2.5\%$ NaCl. Thus, the activity of the starter and its ability to ferment residual lactose is strongly dependent on the S/M level in the curd. *Lactococcus lactis* ssp. *cremoris* is more salt-sensitive than *Lc. lactis* ssp. *lactis*

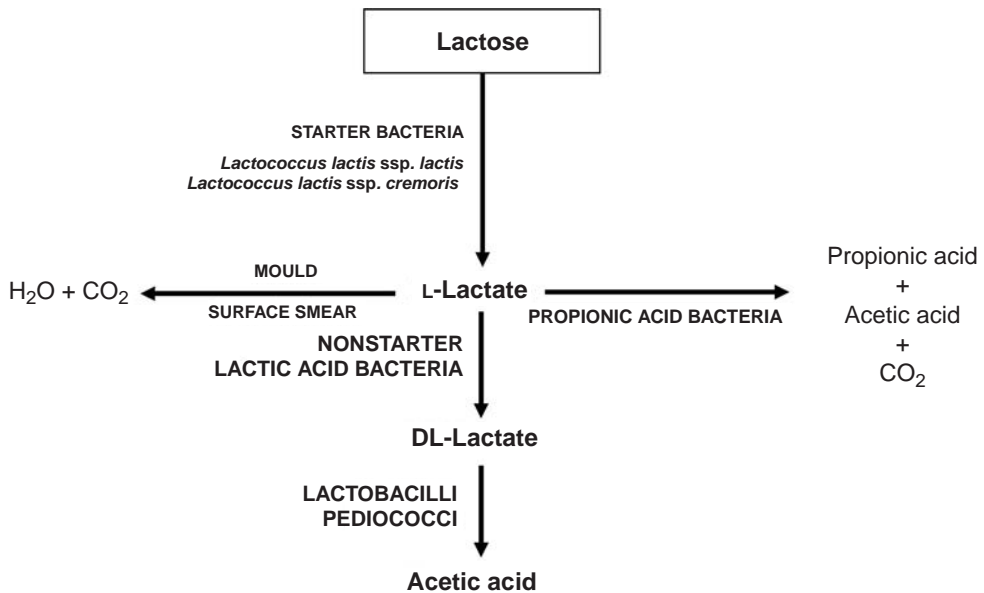


Figure 12.2. Generalized pathways for the metabolism of lactose by mesophilic and thermophilic lactic acid bacteria.

which in turn is more sensitive than NSLAB (Turner and Thomas, 1980) and therefore, the % S/M also determines the products of postmanufacture lactose fermentation.

If starter activity is inhibited after manufacture, residual lactose will be metabolized by nonstarter lactic acid bacteria, mainly pediococci and mesophilic lactobacilli, which are more salt tolerant than starter bacteria and metabolize lactose to DL-lactate and racemize L-lactate. Nonstarter bacteria grow in all cheeses but their growth is markedly dependent on temperature; they have little influence on lactose or lactate concentration until their numbers exceed 10^6 – 10^7 CFU/g (Fox et al., 1990).

The pH at whey drainage largely determines the mineral content of a cheese. The loss of Ca^{2+} and phosphate from casein micelles determines the extent to which they are disrupted and this largely determines the basic structure and texture of a cheese at the end of manufacturing (Lawrence et al., 1983). The recent work of O'Mahony et al. (2005) showed that softening of texture in the first few weeks of ripening was more highly correlated with the level of insoluble calcium than with the level of intact α_{s1} -CN in cheeses early in ripening. This study concluded that the hydrolysis of α_{s1} -CN at Phe²³–Phe²⁴ is not a prerequisite for softening but it is principally due to the

partial solubilization of colloidal calcium phosphate associated with the para- κ -CN matrix of Cheddar cheese during the early stages of ripening. In general, curds with a low pH have a crumbly texture, for example, Cheshire, while high pH curds tend to be more elastic, for example, Emmental.

It has been suggested that the casein in low pH cheese is hydrolyzed more rapidly than in normal pH cheese because depletion of colloidal calcium phosphate from the curd causes micelle dissociation and renders the caseins more susceptible to proteolysis (Fox, 1970; O'Keeffe et al., 1975). Alternatively, more chymosin is retained, and is more active, at low pH (Creamer et al., 1985; Holmes et al., 1977) and this is considered to be mainly responsible for the increased proteolysis in low-pH cheeses (Creamer et al., 1985).

The level of chymosin incorporated in the cheese curd is dependent on the pH at whey drainage (more rennet is retained in the curd at lower pH; Creamer et al., 1985; Holmes et al., 1977; Lawrence et al., 1983) and salt content (Bansal et al., 2007). Lower pH and higher ionic strength leads to reduced electrostatic repulsion between the caseins and chymosin resulting in increased interaction/retention of coagulant in the cheese matrix. The knowledge on the specific interaction(s) involved between the caseins

and chymosin remains largely unknown but from the data available in the literature it seems hydrophobic interaction may play an important role.

Lactic acid is further metabolized by propionic acid bacteria, for example, in the production of Swiss-type cheeses, to propionic acid and CO₂. The production of CO₂ is responsible for the eye formation which is a characteristic of Swiss-type cheeses (Fox et al., 1995). Oxidation of lactate can also occur in cheese. During this process, lactate is converted to acetate and CO₂. Acetate is present at fairly high concentrations in Cheddar and is considered to contribute to cheese flavor, although a high concentration may cause off-flavor (Aston and Dulley, 1982).

Of all cheese varieties, the metabolism of lactate is probably most extensive in the surface mold-ripened cheese, for example, Camembert and Brie. The surface mold (*Geotrichum candidum* and *Penicillium caseicolum*) rapidly metabolize lactate to CO₂ and H₂O, causing an increase in pH. De-acidification occurs initially at the surface, resulting in a pH gradient from the surface to the center and causing lactate to diffuse outwards. When all the lactate has been metabolized, the mold metabolizes proteins, producing NH₃, which diffuses inwards, causing a further increase in pH. The solubility of calcium phosphate decreases with increasing pH, causing precipitation of Ca₃(PO₄)₂ on the surface, thereby causing a calcium phosphate gradient within the cheese. Reduction of the calcium concentration in the interior helps to soften the body of the cheese. The high pH stimulates the action of plasmin, which, together with residual coagulant, is responsible for proteolysis in this cheese rather than proteinases secreted by the surface microflora which, although very potent, diffuse into the cheese to only a very limited extent, although products of their action on the surface proteins may diffuse into the body of the cheese (Fox et al., 1990).

In the case of Blue cheeses, predominantly L⁽⁺⁾-lactate is produced initially. Racemization of L-lactate does occur but lactate does not appear to be metabolized any further (Fox et al., 1990).

The racemization of L-lactate is probably not significant from a flavor viewpoint, but D-lactate may have undesirable nutritional consequences in infants. Calcium D-lactate is believed to be less soluble than calcium L-lactate and may crystallize in cheese, especially on cut surfaces (Dybing et al., 1988). Consumers may mistake the crystals as spoilage, and crystal formation is generally considered negative.

Citrate Metabolism in Cheese. Bovine milk contains relatively low levels of citrate (~8 mM). Approximately, 90% of the citrate in milk is soluble and most of this is lost in the whey; however, the concentration of citrate in the aqueous phase of cheese is ~3 times that in whey (Fryer et al., 1970), presumably reflecting the concentration of colloidal citrate. Cheddar cheese contains 0.2–0.5% (w/w) citrate which is not metabolized by *Lc. lactis* ssp. *lactis* or *Lc. lactis* ssp. *cremoris*, but is metabolized by *Lc. lactis* biovar *diacetylactis* and *Leuconostoc* spp., with the production of diacetyl and CO₂ (Fig. 12.3).

Because of CO₂ production, citrate metabolism is responsible for the characteristic eyes in Dutch-type cheeses. Diacetyl and acetate produced from citrate contribute to the flavor of Dutch-type and Cheddar cheeses (Aston and Dulley, 1982).

Citrate is not metabolized by *Streptococcus salivarius* subsp. *thermophilus* or by thermophilic lactobacilli (Hickey et al., 1983), but several species of mesophilic lactobacilli metabolize citrate with the production of diacetyl and formate (Fryer, 1970); the presence of lactose influences the amount of formate formed.

The principal flavor compounds produced from metabolism of citrate are acetate, diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), and 2,3-butanediol (Cogan, 1995). Diacetyl is usually produced in small amounts but acetoin is generally produced in much higher concentration (10–50 folds higher than diacetyl). Acetate is produced from citrate in equimolar concentrations.

LIPOLYSIS

Cheese is a high-fat food; fresh (acid) cheeses have a fat content up to 12% and ripened cheeses, in general, contain 20–40% fat (Renner, 1993). The fat fraction of cheese is important for the development of typical flavor and good texture. It is well known that a higher fat content leads to a less firm and elastic body, while low-fat products tend to be harder, more crumbly and less smooth than normal (Emmons et al., 1980). In low-fat products, there is increased cross-linking within the curd, which is carried through into the cheese. Increasing the moisture content in an attempt to overcome these defects leads to weak body and encourages an undesirable flavor and poor flavor. The fat fraction of cheese is important for the development of typical flavor. Cheddar cheese made from nonfat milk does not develop full aroma, even after 12 months (Ohern and Tuckey, 1969). Foda et al.

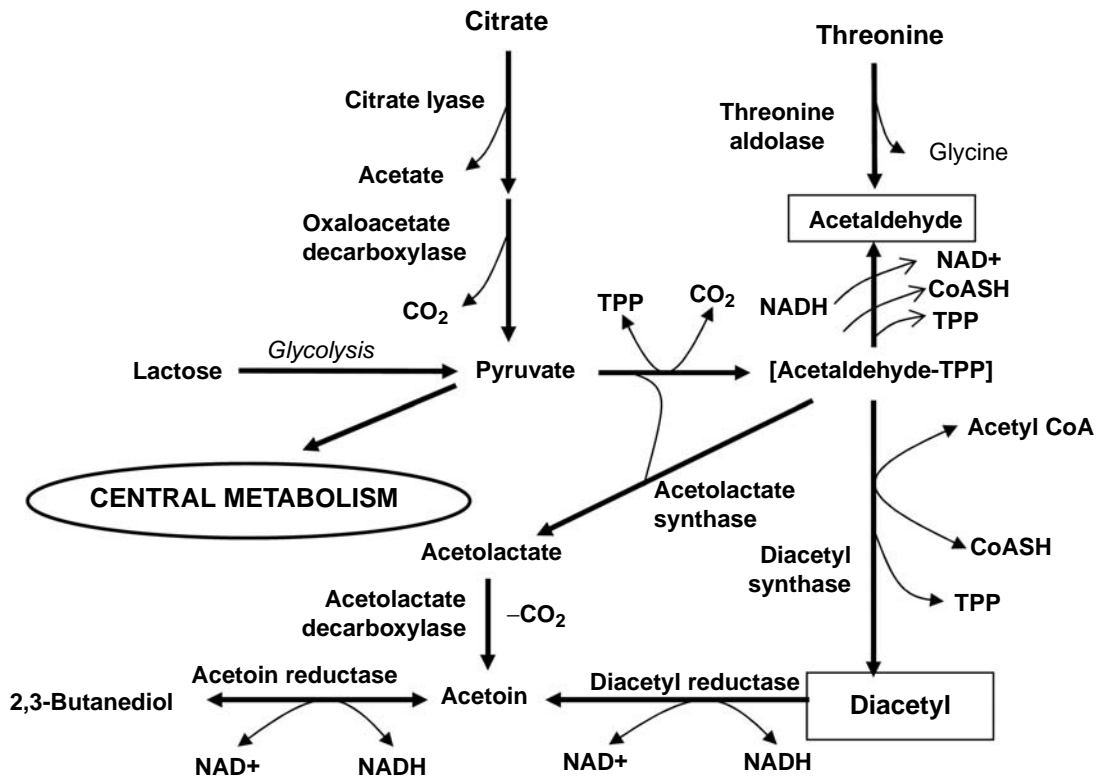


Figure 12.3. Metabolism of citrate by lactic acid bacteria. (Modified from Singh et al., 2003a.)

(1974) also suggested that the fatty acid composition and natural emulsion of milk fat are important for fl vor development.

There has been an increased interest in low-fat cheeses. Cheeses with reasonably good fl vor and texture were successfully made by replacing fat by whey proteins (de Boer and Nooy, 1980; McGregor and White, 1990a,b) and other fat substitutes/mimetics.

Like all types of food with a high-fat content, lipolytic (enzymatic) and oxidative (chemical) changes are likely to occur in cheese. Lipases and esterases in cheese originate from milk, rennet preparation, starter, secondary starter, or NSLAB. A number of psychrotrophic organisms, which can dominate the microflor of refrigerated milk, produce heat-stable lipases. In general, in varieties in which lipolysis is extensive, lipases originate from the coagulant (rennet paste used in Italian varieties contain pre-gastric lipase) or the secondary flor (mould-ripened varieties). Milk contains a well-characterized

indigenous lipoprotein lipase (LPL; Olivecrona et al., 1992), as well as a number of esterases (Deeth and Fitz-Gerald, 1983). The hydrolysis of triglycerides, which constitute more than 98% of milk fat, is the principal biochemical transformation of fat, which leads to the production of free fatty acids (FFA), di- and mono-glycerides, and possibly glycerol (Fig. 12.4).

FFA contribute to the aroma of cheese. Individual FFA, particularly acids between C4:0 and C12:0, have specific fl vors (rancid, sharp, goaty, soapy, coconut-like). The fl vor intensity of FFA depends not only on the concentration but also on the distribution between aqueous and fat phases, the pH of the medium, the presence of certain cations (e.g., Na^+ , Ca^{2+}), and protein degradation products (Adda et al., 1982). The pH has a major influence on the fl vor impact of FFA. At the pH of Cheddar (pH ~5.2), a considerable portion of FFA are present as salts, which are nonvolatile, thus reducing their fl vor impact. In most cheese varieties, relatively little lipolysis occurs

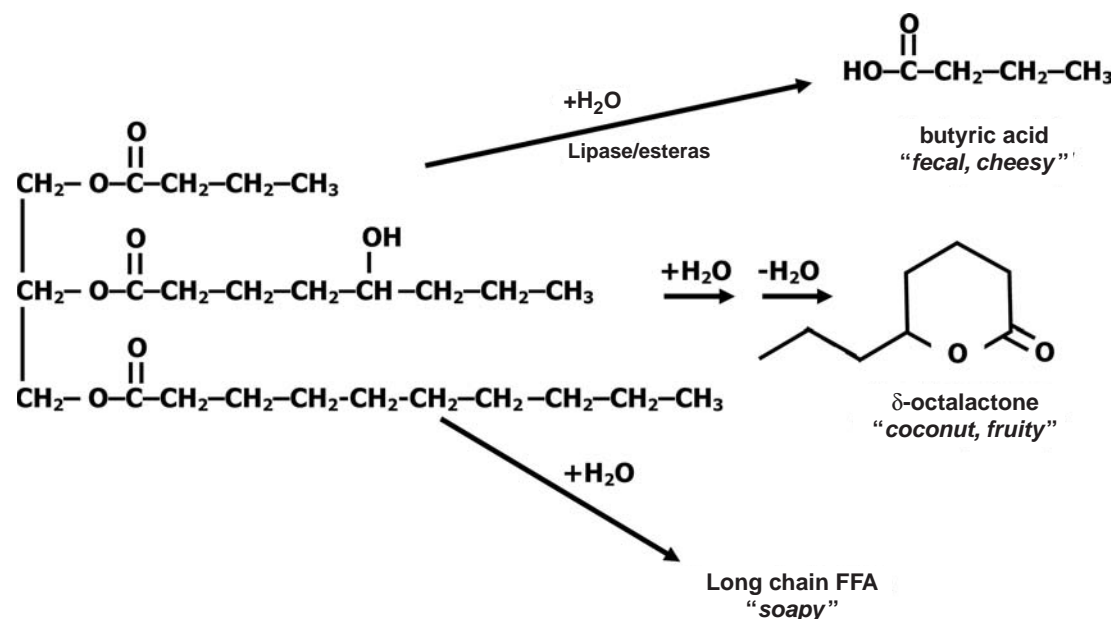


Figure 12.4. Lipolysis of milk triglycerides into cheese flavor compounds.

during ripening and too much is considered undesirable. Most consumers would consider Cheddar-, Dutch-, and Swiss-type cheeses containing even moderate levels of free fatty acids to be rancid. Even lesser amounts of FFA would make fermented milk such as yogurt rancid. The highest levels of lipolysis are observed in traditional mold-ripened cheeses, such as Camembert (Kuzdzal and Kuzdzal-Savoie, 1966) and Roquefort/Blue-veined cheeses (Anderson and Day, 1965). Extensive lipolysis is desirable as part of overall flavor development in certain cheeses, such as hard Italian cheeses (Romano, Provolone), Camembert, Blue, and Feta.

In a recent study, Lopez et al. (2006) used confocal laser scanning microscopy on Emmental cheese to show that the fat globules were mainly disrupted after pressing of curd grains, leading to the release of the milk fat globule membrane (MFGM); fat inclusions were surrounded by pockets of whey, delimited by casein strands. The colonies of bacteria were preferentially localized in situ at the fat/protein interface. This study also showed that both the localization of bacteria and the supramolecular organization of fat which was not protected by the MFGM can help the accessibility of milk fat to lipolytic enzymes and then contribute to the quality of cheese.

Fatty Acids Metabolism

The liberated fatty acids are involved in several types of reaction which vary in importance with the type of cheese involved. The production of methyl ketones follows a two-step reaction: the fatty acids are first oxidized to β -ketoacids, which are then decarboxylated to corresponding methyl ketones with one carbon atom less (Hawke, 1966). Besides this main mechanism, it seems that certain short-chain carbonyl compounds can also result to a limited extent from the metabolic activity of the mold on the indigenous β -ketoacids (Dartley and Kinsella, 1971) which are normally present in small quantities in milk fat. This second mechanism, based on the indigenous β -ketoacids, is the main pathway in cheeses in which mold growth is not involved in ripening (Adda et al., 1982). In cheeses where mold growth occurs, production of methyl ketones is very important. Oxidative degradation is responsible for the formation of $\text{C}_{(n-1)}$ methyl ketones and the resulting secondary alcohols from C_n fatty acids (mainly from $\text{C}_{6:0}$ to $\text{C}_{12:0}$); these compounds are responsible for the characteristic aroma of blue-veined cheeses, but also that of Camembert-type cheeses (Gripon et al., 1991). However, they do play a limited role in Cheddar cheese

fl vor. Ultimately, methyl ketones can be reduced to secondary alcohols, which do not contribute to cheese aroma.

Another reaction in which polyunsaturated and, perhaps, monounsaturated, fatty acids can be involved, is oxidation. The extent of oxidation in cheese is, however, rather limited, possibly due to a low redox potential. This together with the presence of natural antioxidants could prevent the initiation of oxidation mechanisms, or create conditions in which the primary oxidation products are reduced (Adda et al., 1982).

Aliphatic and aromatic esters play an important part in the fl vor and, sometimes, the off-fl vor of cheese. This synthesis mainly concerns the above-mentioned short- or medium-chain fatty acids and the alcohols involved may be aliphatic (ethanol), aromatic (phenylethanol), or thiols (methanethiol). Esters generally contribute a fruity fl vor to dairy products which is desirable and characteristic in many cheeses (Parmesan, Parrano) but undesirable in others (Cheddar).

The mechanism of synthesis of esters in cheese is still largely unknown (Liu et al., 2004b). It is generally accepted that the enzymes of cheese microflor are involved in the formation of cheese esters (Hosono et al., 1974; Liu et al., 2004b), but some authors also suggest that cheese esters are not of enzymatic origin (Adda et al., 1982; Forss, 1972). Most S-methyl thioesters can be formed spontaneously in cheese from the reaction of acyl CoA with methanethiol (Helinck et al., 2000). Two ester-producing reactions could be involved in ester formation in cheese, esterification and alcoholysis. Until recently, ester synthesis in cheese was regarded as resulting from the esterification of an alcohol and an acid. The acid or acyl CoA moieties of esters are formed from the action of the cheese microflor and their enzymes on lactose, lactate, lipids, and proteins of cheese curd (Urbach, 1997a). It has been recently shown that cheese esters could also be synthesized directly from glycerides and alcohols via an alcoholysis reaction. Esterases of LAB can catalyze this reaction, consisting in the transfer of a fatty acyl group from triglycerides (and, preferably, mono- and diglycerides) to an alcohol, without the direct involvement of water (Liu et al., 2003, 2004a). Alcoholysis could be a more common route of esters synthesis in aqueous environments than esterification reaction, which is favored under low water activity conditions (Liu et al., 2004b). The rate-limiting factors of ester synthesis in cheese are unknown. Substrates, en-

zymes, and environment may all determine the rate of ester formation. In Cheddar and Swiss cheeses, however, ethanol is regarded as the limiting factor of ester synthesis (Liu et al., 2004b; Thierry et al., 2006). The "fruitiness" defect of Cheddar cheese, which results from the formation of ethyl esters (Bills et al., 1965), was found significantly correlated to the concentration of ethanol (Manning and Moore, 1979). It was reported that the addition of ethanol to Cheddar curd produced increased levels of ethyl esters (Urbach, 1993). Ethanol is the main alcohol detected in Cheddar cheese (McGugan et al., 1975). Its formation in Cheddar cheese mainly results from the activity of heterofermentative lactobacilli and/or yeasts. A recent study by Thierry et al. (2006) demonstrated that ethanol is the limiting factor of ethyl ester synthesis in Swiss cheese.

Lactones (γ - and δ -) are potent fat-derived fl vor compounds that play an important role in the overall cheese fl vor profile particularly in Cheddar (Drake et al., 2001; Wong et al., 1973). Data from a recently published work (Alewijn et al., 2006) showed that lactic acid bacterial enzymatic activities played no role in the formation of lactones from milk triglycerides or free fatty acids. The same study also demonstrated that the mechanism of lactone formation in cheese is a one-step, nonenzymatic reaction, where a hydroxyl fatty acid esterified in a triglyceride undergoes *trans*-esterification to release the lactone directly.

Proteolysis

During the manufacture and ripening of cheese, a gradual decomposition of caseins occurs due to the combined action of various proteolytic agents. These generally include enzymes from following sources:

- Coagulant
 - a) Chymosin (genetically engineered/fermentation derived)
 - b) Chymosin/pepsin (from calf stomach)
 - c) Chymosin substitutes-microbial (e.g., *Rhizomucor miehei*, *Endothia parasitica*)
 - d) Plant (e.g., *Cynara cardunculus*)
- Indigenous milk enzymes (e.g., plasmin, cathepsin)
- Starter and nonstarter bacterial enzymes
 - a) Cell envelope-associated proteinases (Lactocypins)

- b) Peptidases (e.g., endopeptidases, aminopeptidases, di- /tri-peptidases and proline-specific peptidases
- Secondary starter enzymes
- Exogenous enzymes added during cheese making as ripening aid

Enzymes from the first three sources are active in most ripened cheeses. The secondary starter (i.e., microorganisms added to cheese milk or curd for purposes other than acidification) exerts considerable influence on the maturation of cheese varieties in which they are used (e.g., *Penicillium roqueforti* or *P. camemberti* in mold-ripened varieties or *Brevibacterium linens* in smear-ripened cheeses). Exogenous enzymes used to accelerate ripening, when present can be very influential.

The correct pattern of proteolysis is generally considered to be a prerequisite for the development of the correct flavor of cheese. Products of proteolysis *per se* (i.e., peptides and free amino acids) probably are significant in cheese taste, at least to "background" flavor, and some off-flavors, for example, bitterness, but are unlikely to contribute much to aroma. Compounds arising from the catabolism of free amino acids contribute directly to cheese taste and aroma.

The contribution of the above enzymes, individually or in various combinations, has been assessed using three complementary approaches:

- (1) Model cheese systems from which the nonstarter microflora have been eliminated by aseptic techniques, in which acidification is accomplished by an acidulant (usually glucono- δ -lactone) rather than starter, and in which coagulant and indigenous milk enzymes may be inactivated or inhibited;
- (2) Activity and specificity of the principal proteinases and peptidases on caseins or casein-derived peptides in solution; and
- (3) Isolation of peptides from cheese and, based on the known specificity of the proteinases/peptidases on the caseins in solution, identification of the agent(s) responsible for their formation in cheese (Fox et al., 1994).

In most cheese varieties, the coagulant is responsible for the initial hydrolysis of caseins, for example, as shown by PAGE and the formation of water- or pH 4.6-soluble N (Visser, 1976, 1977a, b, c; Visser and de Groot-Mostert, 1977). Its action is restricted largely to α_{s1} -casein, with little or no hydrolysis of β -casein and probably not of α_{s2} -casein. Indigenous milk and

starter proteinases are less important at this level of proteolysis, but the production of small peptides and amino acids is due primarily to the action of starter bacteria or their enzymes.

The principal indigenous milk proteinase, plasmin, appears to be mainly responsible for the relatively limited proteolysis of β -casein in Cheddar and Dutch-type cheeses but is more significant in high-cooked cheeses (e.g., Swiss types), in which chymosin is extensively or completely inactivated (Visser, 1993).

The starter lactic acid bacteria (*Lactococcus lactis* ssp.) possess a very comprehensive proteolytic system (Kunji et al., 1996). Aseptic starter-free cheeses, containing normal amounts of rennet, show the development of high levels of soluble N, indicating the importance of rennet for soluble N production. However, the production of soluble N in these cheeses is less than in normal aseptic cheeses, indicating that in addition to rennet (and plasmin); the starter bacteria also contribute to the production of soluble N. Significant amounts of soluble N were produced in aseptic rennet-free cheeses, suggesting that starter bacteria are capable of attacking para-casein in cheese and converting it to soluble products, independently of rennet action (Visser, 1977c). Studies undertaken to elucidate the role of lactococcal proteinases in proteolysis in Cheddar cheese include comparison of proteolysis in cheese made with lactococcal starters and their proteinase-negative derivatives (Broome et al., 1991; Farkye et al., 1990; Law et al., 1993; Oberg et al., 1986). Broome et al. (1991) reported that the loss of proteinase activity in starter (Prt⁻ starter) reduced overall proteolysis by approximately 50%, which clearly shows a more important role for starter bacteria in the initial degradation of milk protein than previously thought.

Nonstarter lactic acid bacteria (predominantly mesophilic lactobacilli) usually dominate the microflora of Cheddar-type cheese during much of its ripening. Nonstarter lactic acid bacteria possess a wide range of proteolytic enzymes (Atlan et al., 1993) and may contribute to the formation of short peptides and free amino acids in Cheddar. Broome et al. (1991) reported that the main effect of adding *Lb. casei* to Prt⁻ starter cheese was to increase peptidase activity which resulted in PTA-soluble N and amino acid levels similar to the Prt⁺ control cheese. Lynch et al. (1994) prepared cheeses under aseptic conditions from milk containing selected species of mesophilic lactobacilli as adjunct starter. No differences were observed between control and experimental cheeses

in term of flavor and texture but experimental cheeses showed higher levels of free amino acids, again indicating increased peptidase activity due to lactobacilli.

The contribution of individual agents varies substantially between varieties, for example, in Mozzarella, Swiss, and other high-cook varieties, the coagulant is extensively or completely denatured and the contribution of plasmin to initial proteolysis is more pronounced than in Cheddar and Dutch varieties. In mold or bacterial surface-ripened varieties, proteinases and peptidases from the secondary microflora influence proteolysis strongly.

Proteolysis in Cheese. The extent of proteolysis varies from very limited (e.g., Mozzarella) to very extensive (e.g., Blue cheese) and the resulting products range from water-insoluble polypeptides, comparable in size to intact caseins, through water-soluble intermediate-sized and small peptides to free amino acids (Fox et al., 1994).

Proteolytic degradation of caseins into peptides in Cheddar (Fernandez et al., 1998; McSweeney et al., 1994, Singh et al., 1994, 1995, 1997), Emmentaler (Gagnaire et al., 2001), and Parmigiano-Reggiano cheeses (Addeo et al., 1992, 1994) were characterized in detail.

Hydrolysis of α_s -casein. In the cheese environment, with a high ionic strength and a low a_w , rennet-induced breakdown of α_{s1} -casein (CN) proceeds much faster than that of β -CN (α_2 - and κ -CNs are quite resistant to hydrolysis by the rennet; Visser, 1993). The residual chymosin rapidly hydrolyses α_{s1} -CN at the bond Phe²³-Phe²⁴ during the initial stages of ripening (Creamer and Richardson, 1974). The peptide α_{s1} -CN f1-23, produced by chymosin action on the bond Phe²³-Phe²⁴ of α_{s1} -casein, is further hydrolyzed in Cheddar cheese (Singh et al., 1994) by CEP from starter *L. lactis* ssp. *cremoris* resulting in the production of whole range of small molecular weight peptide. Two small peptides from α_{s1} -CN f1-23, namely α_{s1} -CN f1-9 and α_{s1} -CN f1-13, were found to accumulate in Cheddar during ripening (Singh et al., 1994). Kaminogawa et al. (1986) also reported the accumulation of α_{s1} -CN f1-9, f1-13 and f1-14 in Gouda cheese. The small peptides from α_{s1} -CN f1-23 representing N-terminal (α_{s1} -CN f1-7, 1-9, 1-13, and 1-14) and C-terminal (α_{s1} -CN f14-17, 17-21) sequences were found to be bitter in taste (Lee et al., 1996, Richardson and Creamer, 1973). Chymosin produced large peptide α_{s1} -CN f24-199 is

further hydrolyzed by chymosin and CEP (Fig. 12.5a; for further details see McSweeney et al., 1994; Singh et al., 1995, 1997).

Relatively few peptides originating from α_{s2} -CN have been identified in Cheddar (Fernandez et al., 1998; Singh et al., 1995, 1997), Emmentaler (Gagnaire et al., 2001), and Parmigiano-Reggiano (Addeo et al., 1992, 1994) cheeses. This limited data showed that plasmin played an important role in the initial degradation of this protein into intermediate size peptides. Plasmin-produced peptides were further hydrolyzed into small peptides and amino acids mainly by the proteinases and peptidases of the starter and NSLAB.

Hydrolysis of β -casein. Chymosin has limited action on β -CN in Cheddar, although some activity is indicated by the presence of the peptide β -CN f1-192 (McSweeney et al., 1994). Hydrolysis of the bond Leu¹⁹²-Tyr¹⁹³ of β -CN by chymosin releases a small corresponding C-terminal fragment, β -CN f193-209, which is extremely bitter (Singh et al., 2004b). Hydrolysis of β -CN is strongly inhibited by 5% NaCl and completely by 10% NaCl (Fox and Walley, 1971; Thomas and Pearce, 1981). Interestingly, chymosin hydrolyzes β -CN at a much faster rate than α_{s1} -CN in a solution of Na-caseinate in distilled water (Wang and Fox, personal communication). Increasing the ionic strength of the solution dramatically reduces the sensitivity of β -CN to chymosin (Hunter and Fox, personal communication). From these studies, it can be concluded that the effect of salt on the hydrolysis of β -CN is due to the modification of substrate rather than of the enzyme (Mulvihill and Fox, 1978). Increasing the ionic strength of β -CN solution promotes intermolecular hydrophobic interactions between its hydrophobic C-terminal region which contains the chymosin-sensitive bonds (Fox, 1989). During the ripening of Cheddar cheese, β -CN undergoes limited hydrolysis by plasmin (Creamer, 1975) but does not appear to be hydrolyzed by chymosin (Fox and Walley, 1971). In a study (Kelly, 1993) on the influence of salt on proteolysis during the ripening of Cheddar, it was shown that cheese made with no added salt developed bitterness, but this defect did not occur in cheeses containing an increasing level of salt.

Nearly half of the β -CN in Cheddar cheese is hydrolyzed during the ripening. Plasmin, an indigenous milk proteinase, is mainly responsible for the initial proteolysis of this protein. Plasmin hydrolysis of β -CN results in the formation of three γ -CNs [γ_1 - (β -CN f29-209), γ_2 - (β -CN f106-209), and

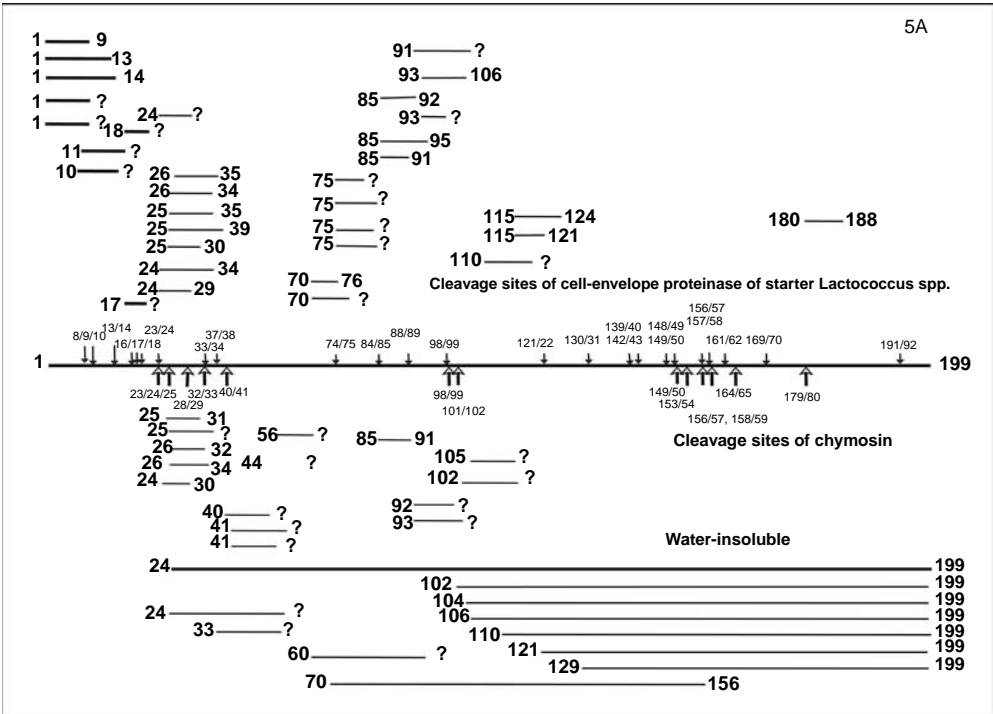


Figure 12.5. Hydrolysis of α_{s1} - (a) and β -caseins (b) in Cheddar cheese during ripening. ? Indicated C-terminal end not definitely determined. (Redrawn from Fernandez et al., 1998; McSweeney et al., 1994; Singh et al., 1994, 1995, 1997.)

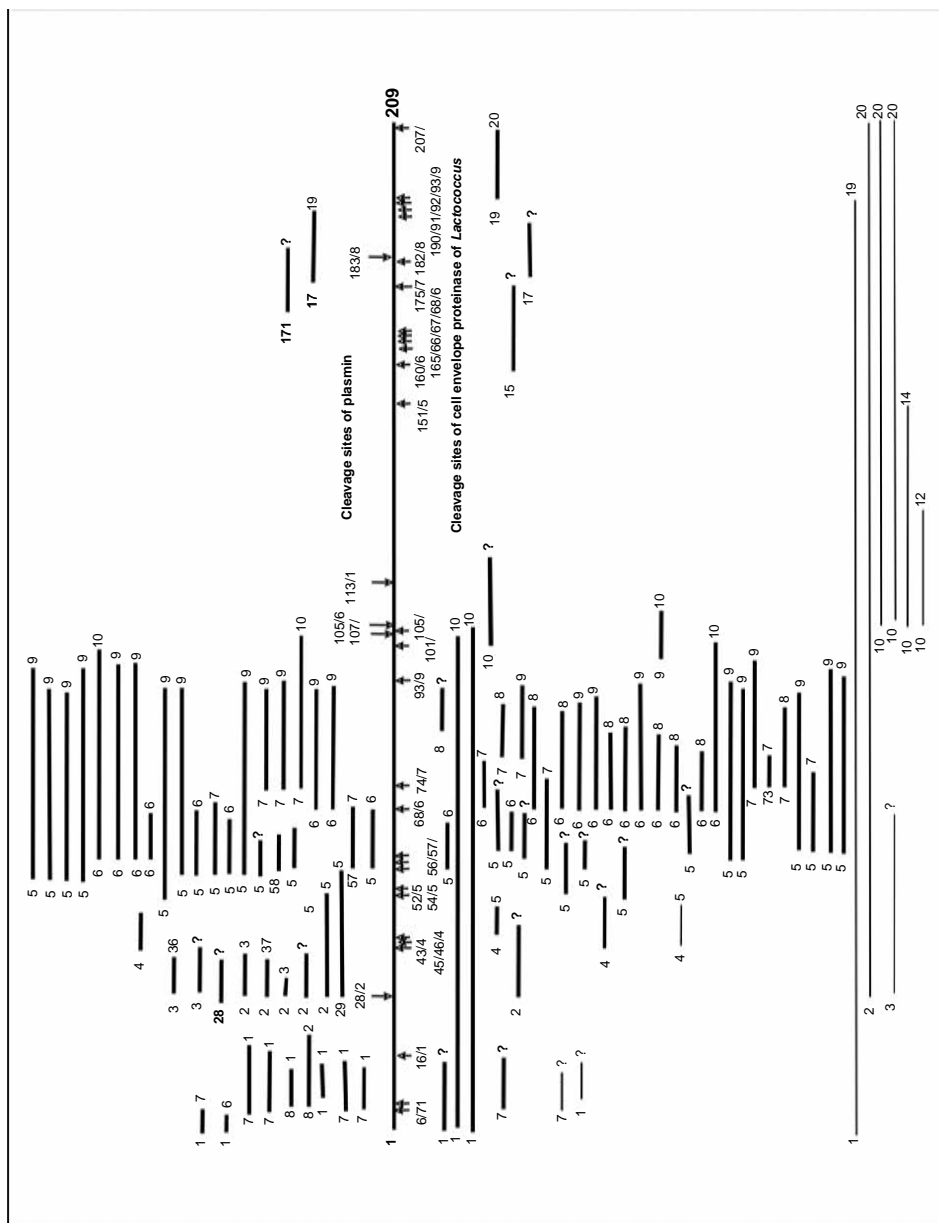
γ_3 - (β -CN f108-209) caseins], representing C-terminal region, and fi e proteose-peptones [β -CN f1-28, β -CN 1-105/107, and β -CN f29-105/107] representing the corresponding N-terminal region. The γ -CNs seems to accumulate in Cheddar over the ripening period. The amount of γ -CNs in Cheddar increases during ripening (Farkye and Fox, 1992) but the increase is more pronounced in Gouda (Visser and de Groot-Mostert, 1977) and Emmental (Ollikainen and Kivela, 1989). The slightly higher pH and moisture content in Gouda might be responsible for the slightly higher plasmin activity. Washing of cheese curd during manufacture may remove inhibitors of plasmin or plasminogen-activator, thus increasing plasmin activity. A high cooking temperature enhances plasmin activity in cheese, which can probably be attributed to an increased conversion of plasminogen to plasmin under those conditions (Visser, 1993). The high cooking temperature ($>50^{\circ}\text{C}$) used in the manufacture of Swiss-type cheeses largely inactivates the residual chymosin in the curd and there-

fore accentuates the relative importance of plasmin in the ripening of these cheeses (Ollikainen and Kivela, 1989; Richardson and Pearce, 1981). Plasmin also contributes significantly to the ripening of cheese, the pH of which increases during ripening, for example, Camembert.

The proteose-peptones are extensively hydrolyzed by the starter bacterial CEP and peptidases to produce small peptides and free amino acids. Most of the β -CN-derived peptides identified in Cheddar originated from the proteose-peptones (Singh et al., 1995, 1997; Fig. 12.5b).

A hydrophobic peptide (β -CN f58-72) identified in Cheddar was found to inhibit intracellular endopeptidase, Pep X and Pep N (Stepaniak et al., 1995); these results demonstrate that cheese ripening may be influenced by inhibitory peptides originating from β -casein.

In Cheddar, Parmigiano-Reggiano and Emmentaler cheeses, large numbers of peptides derived from the region 1-107 of β -CN have been identified and



shown to accumulate during the ripening of cheese (Addeo et al., 1992, 1994; Gagnaire et al., 2001; Singh et al., 1995, 1997). In addition to their nutritive properties, caseins, in particular β -CN, are also the source of peptides with biological activity, such as reducing blood pressure (antihypertensive), opioid activity, and phosphopeptides. Quite diverse range of biological activities were found to originate from region 50–80 of β -CN, for example, angiotensin-converting enzyme (ACE) inhibition, opioid activity, starter bacterial peptidase inhibitor (Donkor et al., 2007; Gobbetti et al., 2002).

Proteolysis in cheese seems to be a sequential process involving rennet, milk proteinase (particularly plasmin), the starter culture, secondary microorganisms, and NSLAB:

- The hydrolysis of casein to high molecular weight peptides is thought to be primarily the result of chymosin and plasmin
- The subsequent hydrolysis of high molecular weight peptides, into small peptides and free amino acids, is primarily the result of proteolytic enzymes from LAB (Singh et al., 2003a)

Amino Acids Metabolism. Amino acid degradation plays a vital role in flavor development in cheese. A number of researchers have attempted to enhance free amino acid content in Cheddar cheese by direct addition of amino acids (Wallace and Fox, 1997) and genetic modification of lactococci with increased aminopeptidase N activities (Christensen et al., 1995; McGarry et al., 1994). But increased amino acid content in Cheddar did not affect the flavor development, which led Yvon et al. (1998) to hypothesize that the rate-limiting factor in flavor biogenesis was not the release of amino acids but their subsequent conversion to aroma compounds. Yvon et al. (1998) identified transaminase acceptor α -ketoglutarate as the first limiting factor in degradation of amino acid. Addition of α -ketoglutarate to Cheddar curd resulted in increased volatile components originating from branched chain and aromatic amino acids (Banks et al., 2001).

In lactococci, the first step in the degradation of amino acids is transamination (Fig. 12.6; Gao et al., 1997), leading to formation of α -keto acids (α -KA).

Aromatic aminotransferase enzymes have been previously characterized from *L. lactis* ssp. *cremoris* (Rijnen et al., 1999a; Yvon et al., 1997) and *L. lactis* ssp. *lactis* (Gao and Steele, 1998). These enzymes initiate the degradation of Val, Leu, Ile, Phe, Tyr, Trp,

and Met, all of which are known precursors of cheese flavor compounds. Inactivation of aminotransferase enzymes involved in the breakdown of amino acids by lactococci has been shown to reduce aroma formation during cheese ripening (Rijnen et al., 1999b).

α -Keto acids are central intermediates, and can be converted to hydroxyl acids, aldehydes, and CoA-esters (Fig. 12.7).

These reactions are mostly enzymatic, but some chemical conversion steps have also been described, like the formation of benzaldehyde from phenylpyruvic acid (Smit et al., 2004). The aldehydes formed can generally be dehydrogenated or hydrogenated to their corresponding alcohols or organic acids, which are in their turn substrates for esterases and acyltransferases leading to (thio)esters (Fig. 12.6).

Other enzymatic route for the conversion of amino acids involved lyases (e.g., cystathionine β -lyase, threonine aldolase) and deimination/decarboxylation (resulting in the formation of amines). For further details see Smit et al. (2005).

The volatile fraction of cheese has several sulfur-containing compounds such as methanethiol, methional, dimethyl sulfide dimethyldisulfide dimethyltrisulfide dimethyltetrasulfide carbonyl sulfide and hydrogen sulfide (Lindsay and Rippe, 1986; Urbach, 1995; Weimer et al., 1999) and they contribute to the aroma of cheese (Milo and Reineccius, 1997). These compounds are known to originate from Met (Fig. 12.7). Methanethiol is readily oxidized to dimethyl disulfide and dimethyl trisulfide (Chin and Lindsay, 1994; Parliament et al., 1982). Occurrence of these compounds is a direct result of methanethiol content and is modulated by the low redox potential present in cheese. Methanethiol can potentially oxidize during analysis to form these compounds, and this may account for some reports of dimethyl disulfide and dimethyl trisulfide in cheese. Dimethyl sulfide (Milo and Reineccius, 1997) and dimethyl trisulfide were recently noted as important odorants in aged Cheddar cheese (Milo and Reineccius, 1997; Suriyaphan et al., 2001b; Zehentbauer and Reineccius, 2002). Further work is needed to define the mechanism and cheese conditions needed for production.

Results of a recent study showed that Cheddar cheese made using adjunct starter *Lactobacillus casei* (genetically modified to enhance expression of hydroxyl acid dehydrogenase, HADH) retarded the flavor development (Broadbent et al., 2004). HADH catalyzes conversion of α -keto acids to α -hydroxy acids, which has little or no importance from flavor

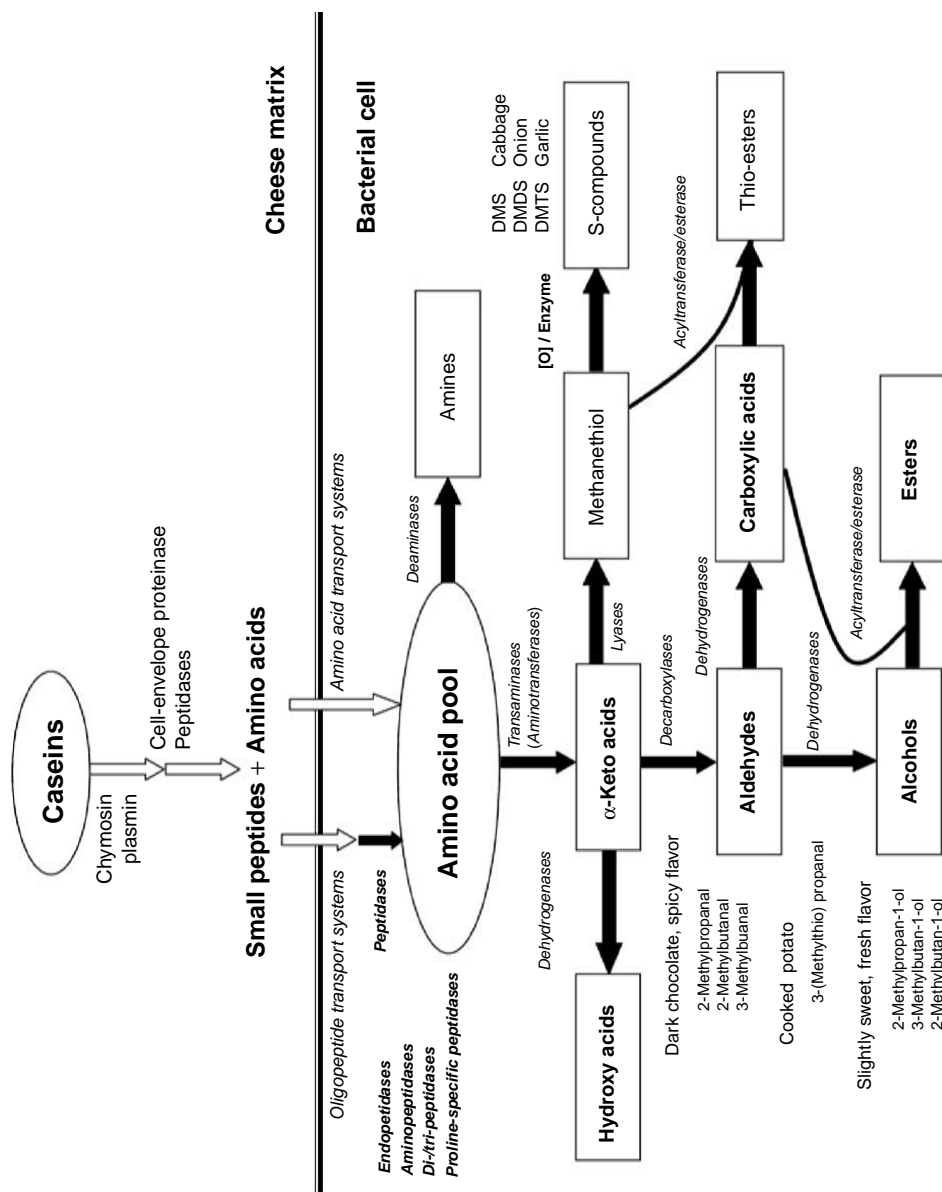


Figure 12.6. Generation of flavor compounds from milk protein degradation. DMS, dimethyl sulfide; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide. (Modified from Singh et al., 2003a.)

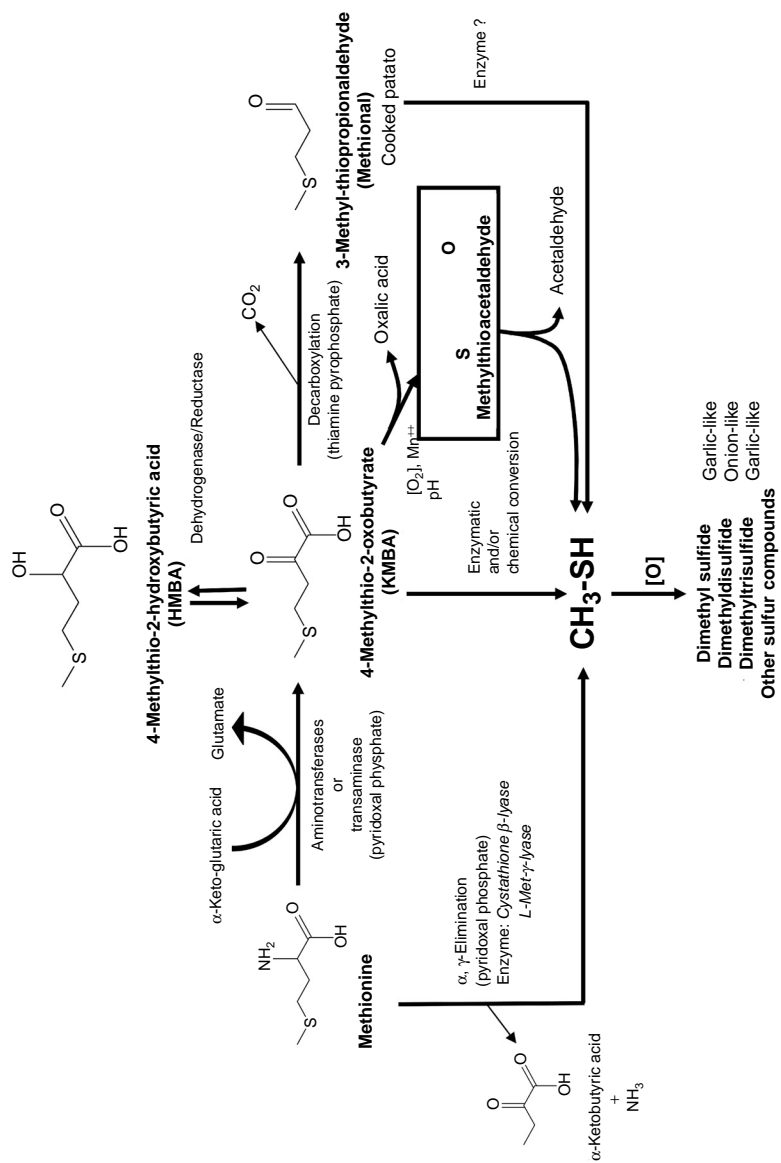


Figure 12.7. Degradation of methionine into potent sulphur containing odorants. (Modified from Singh et al., 2003a.)

point of view. But it may still be possible to selectively suppress aromatic amino acid (Phe, Tyr, Trp)-derived off-flavor compounds by over expression of an alternative HADH with more narrow specificity for aromatic amino acid-derived α -keto acids.

The detailed understanding mechanistic pathways involved in the degradation of free amino acids into cheese flavor compounds and volatile generation capabilities of starter/nonstarter bacteria will not only result in enhanced control/acceleration of cheese flavor but also in minimizing off-flavor developments.

CHARACTERIZATION OF KEY AROMA IMPACT COMPOUNDS OF CHEESES

There has been extensive research on the flavor of cheeses, but despite this effort, only limited information is available on the chemistry of flavor of most cheese varieties and the key flavor compounds of few varieties has been characterized (McGorin, 2001; Parliament and McGorin, 2000; Singh et al., 2003a, 2007). But the flavor chemistry of none is characterized sufficiently to permit its reproduction by mixtures of pure compounds in a cheese model. This may be due to limited information available on the flavor/matrix interaction and taste compounds. Cheeses in many previous studies were simply analyzed for flavor by cheese graders. Such qualitative sensory data has limited use. More defined and analytical information using descriptive sensory and instrumental analysis is required. In the last couple of decades, a number of published works attempted to characterize the mechanism/enzymology of various reactions involved in generation of volatiles in cheese. Only recent work in the last decade has attempted to study cheese flavor in detail.

The availability of powerful chromatographic separation techniques like high-resolution capillary gas chromatography in combination with mass spectrometry and olfactory detection techniques (use of olfactory detection ports), has revolutionized the work on characterization of flavor compounds. Advancements in instrumental/chemical analysis have paralleled the developments in sensory methods for the analysis of flavor compounds. Recently, published reviews by Parliament and McGorin (2000), McGorin (2001), Singh et al. (2003a, 2007), Cadwallader (2007), and Drake et al. (2007b) described various sensory-directed analytical flavor techniques used in

the evaluation of key aroma compounds in milk and dairy products. A recently published text on *Sensory-Direct Flavor Analysis* edited by Marsili (2007a) is particularly recommended to readers who may be interested in more details on flavor analysis techniques. In recent years key odorants in a number of cheese varieties have been characterized by GCO (gas chromatography olfactometry) and aroma extract dilution analysis (AEDA).

AROMA IMPACT COMPOUNDS OF FETA AND PASTA FILATA CHEESES

Ultrafiltration concentration of milk in large industrial-scale cheese manufacturing has been successfully adapted for Feta cheese production. Flavor profile of Feta is dominated by butanoic acid, hexanoic acid, acetic acid, and esters (Horwood et al., 1981b).

Pasta filata cheeses traditionally originate from Southern Italy, and include soft or semisoft varieties consumed as fresh products (e.g., Mozzarella and Scamorza), or semihard and hard varieties, which are ripened before being consumed (e.g., Caciocavallo, Ragusano, and Provolone). Pasta filata cheeses are manufactured in two steps: curd making followed by cooking/stretching. Volatile data on many pasta filata-type cheeses are reported in the literature but in nearly all of those publications authors have made no attempt to characterize cheese flavor by detailed sensory and instrumental analysis. Key flavor compounds in Mozzarella cheese were identified by Moio et al. (1993) by GC-O-MS using AEDA. Mozzarella cheeses produced using buffalo or cow milk were found to have very different volatiles compounds in their aroma profile (Table 12.1).

AROMA IMPACT COMPOUNDS OF GOAT MILK CHEESE

Numerous (>80) odor-active compounds were identified in fresh Chevre-style goat cheese and assessed by sensory analysis of model cheeses for their specific role in the overall aroma. Overall, flavor was found to be dominated by 2,3-butanedione, 1-octen-3-one, *o*-aminoacetophenone, lactones, and octanoic acid. In addition, 4-methyl octanoic and 4-ethyl octanoic acids were found impart waxy/crayon odor to fresh goat cheese (Carunchia-Whetstone et al., 2003).

Table 12.1. Aroma Impact Compounds Identified in Mozzarella Cheese

Mozzarella (Bovine Milk) ^a	Mozzarella (Water Buffalo Milk) ^a
Ethyl-3-methyl butanoate	1-Octen-3-ol
Ethyl isobutanoate	Nonanal
2/3-Methyl-1-butanol	Indole
Phenyl acetaldehyde	3-Hydroxy-2-butanone
Ethyl hexanoate	3-Methyl-2-buten-1-ol
Ethyl butanoate	2-Octanone
Nonanal	2-Hydroxy-3-pentanone
1-Octen-3-ol	Heptanal

^a Mozzarella cheese analyzed by GCO/AEDA/GC-MS (Moio et al., 1993).

Aroma Impact Compounds of Mold Ripened Cheeses

Odorants in surface mold ripened cheeses like Camembert were studied in detailed by Kubickova and Grosch (1997, 1998a,b; Table 12.2).

Aroma compounds were analyzed by GCO using both AEDA and aroma extract concentration analy-

sis (AECA). Compounds like 1-octen-3-ol and the corresponding ketone were found to be responsible for the mushroom/musty aroma note of Camembert. Kubickova and Grosch (1998a) incorporated key odorants identified in Camembert in a model cheese, which was found to be close to the genuine Camembert. Origins and properties of compounds involved in the flavor of surface ripened cheeses was reviewed by Molimard and Spinnler (1996). Gorgonzola is an Italian soft blue-veined cheese made from whole cows' milk inoculated with spores of *Penicillium roqueforti* var. *Weidemannii*. There are two commercial types of Gorgonzola cheese, a traditional variety, also termed "natural," and a "creamy" type also called "sweet," with a more delicate taste and less pungent flavor. 1-Octen-3-ol, ethyl hexanoate, 2-nonanone, 2-heptanone, 2-heptanol, ethyl butanoate, 2-nonanol, and 4-methylanisole were the key odorants of the natural cheese, whereas 2-heptanone, 2-heptanol, ethyl butanoate, 3-(methylthio) propanal, and an unidentified constituent with a fruity odor were characteristic of the creamy Gorgonzola cheese (Moio et al., 2000; Table 12.2). On the basis of high odor activity values, 2-nonanone, 1-octen-3-ol, 2-heptanol, ethyl hexanoate, methylanisole, and 2-heptanone were the most important odorants of natural and creamy Gorgonzola cheese aroma (Moio et al., 2000).

Table 12.2. Aroma Impact Compounds Identified in Mould Ripened Cheeses

Blue-Type ^a	Gorgonzola (Normal) ^b	Gorgonzola (Sweet or Creamy) ^b	Camembert ^c
2,3-Butanedione	1-Octen-3-ol	2-Heptanone	2,3-Butanedione
2-Methyl propanal	Ethyl hexanoate	2-Heptanol	3-Methyl butanal
3-Methyl butanal	2-Nonanone	Ethyl butanoate	3-(Methylthio) propanal
Ethyl butanoate	2-Heptanone	3-(Methylthio) propanal	1-Octen-3-ol
Ethyl hexanoate	2-Heptanol	Unknown (fruity odor)	1-Octen-3-one
3-(Methylthio) propanal	Ethyl butanoate		Phenyl ethyl acetate
Dimethyl trisulfid	2-Nonanol		2-Undecanone
2-Heptanone	4-Methylanisole		δ-Decalactone
2-Nonanone			Methanethiol
			Dimethyl sulfid
			Acetaldehyde
			Hexanal
			Dimethyl trisulfid
			Butanoic acid
			Isovaleric acid

^a Blue cheese analyzed by GCO/AEDA/GC-MS (Qian et al., 2002).
^b Gorgonzola cheese analyzed by GCO/AEDA/GC-MS (Moio et al., 2000).
^c Camembert cheese analyzed by GCO/AEDA/AECA/GC-MS and GCO-H (Kubickova and Grosch, 1997).

Table 12.3. Aroma Impact Compounds Identified in Swiss-Type Cheeses

Swiss Gruyere ^a	Emmentaler Cheese ^b
2/3-Methyl butanal	3-(Methylthio) propanal
3-(Methylthio) propanal	HDMF
Dimethyl trisulfid	Ethyl furaneol
Phenyl acetaldehyde	Acetic acid
2-Ethyl-3,5-dimethylpyrazine	Propanoic acid
2,3-Diethyl-5-methylpyrazine	
Methanethiol	
Acetic acid	
Propanoic acid	
Butanoic acid	
3-Methyl butanoic acid	
Phenyl acetic acid	

^a Swiss Gruyere cheese analyzed by GCO/AEDA/GC-MS and DH-GC-MS (Rychlik and Bosset, 2001a,b).

^b Key Emmentaler cheese odorants identified by the calculation of odor activity values (Preininger and Grosch, 1994).

Aroma Impact Compounds of Swiss Emmentaler Cheese

Key odorant in Swiss Emmentaler aroma profile were methional, HDMF, ethyl furaneol, diacetyl, 3-methyl butanal, and esters (see Table 12.3; Preininger and Grosch, 1994).

Cheese models composed of methional, HDMF, ethyl furaneol, acetic acid, propanoic acid, lactic acid, succinic acid, glutamic acid, sodium, potassium, calcium, magnesium, ammonium, phosphate, and chloride were judged to match the flavor of Swiss Emmentaler cheese very well (Preininger et al., 1996). The flavor of typical Swiss Gruyere cheese (Table 12.3) and a Gruyere sample exhibiting a potato-like off-flavor were characterized by Rychlik and Bosset (2001a,b). Odorants like methional, 2-ethyl-3, 5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine were the probable source of potato-like off-flavor in Gruyere. The flavor of Gruyere cheese was described as more intensely sweaty as compared to Emmentaler cheese (Muir et al., 1995). These differences were possibly due to the lower concentration of caramel-like smelling HDMF and ethyl furaneol

and the higher concentration of 2-/3-methyl butanoic acids in Gruyere as compared to Emmentaler cheese (Preininger et al., 1996; Rychlik and Bosset, 2001b).

Key odorants in Emmentaler cheese were identified by Preininger and Grosch (1994; see Table 12.3). Thierry et al. (1999) studied production kinetics of the key odorants in Emmentaler during ripening. Headspace compounds in Emmentaler cheese aqueous phase showed that alcohols and esters markedly increased in number and concentration, during the ripening in the warm room, to a lesser extent, sulfur compounds, methyl ketones, and 3-methylbutanal, whereas the other aldehydes decreased.

Aroma Impact Compounds of American style Muenster Cheese

Muenster cheese made in United States, using *Streptococcus thermophilus* as culture and no surface smear, was described by cooked/milky, whey, milk fat/lactone, sour, and salty notes using descriptive sensory analysis. The use of dynamic headspace dilution analysis (DHDA) methodology, previously described by Cadwallader and Baek (1998), showed that the most aromatic compounds in the headspace of Muenster were 2,3-butanedione, dimethyl sulfide dimethyl disulfide 2/3-methylbutanal, and 2-acetyl-2-thiazoline (Singh et al., 2003b).

Aroma Impact Compounds of Cheddar Cheese

Flavor of Cheddar cheese is by far the most widely studied. It is generally accepted that the flavor quality of Cheddar cheese in the marketplace today differs considerably from that manufactured before the wide use of pasteurization, microbial rennets, and other modern manufacturing practices (Dunn and Lindsay, 1985). Much of the differences between traditional and contemporary Cheddar flavors probably should be attributed to current marketing of bland-flavored young cheeses. However, even longer aged cheeses are frequently criticized for a lack of adequate Cheddar-type flavor.

In order to evaluate important odorants, GCO/AEDA was first applied to Cheddar cheese by Christensen and Reineccius (1995). The components found to have the highest potency (dilution factor) in a 3-year-old Cheddar cheese were ethyl acetate, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, α -pinene, ethyl butyrate, ethyl caproate, 1-octen-3-one, acetic acid, methional, propanoic acid, butanoic

acid, pentanoic acid, hexanoic acid, decanoic acid, and dodecanoic acid. The authors pointed out that the technique did not allow the determination of the most volatile odor fraction, which included hydrogen sulfide, acetaldehyde, and methanethiol. Descriptive sensory analysis was not conducted on the cheese used in the study, which limited conclusions about the role of individual compounds on specific cheese flavors. On the basis of these results, a subsequent sensory study using a concept matching technique was conducted. Dacremont and Vickers (1994) found that a recognizable Cheddar aroma was produced by a mixture of 2,3-butanedione, methional, and butanoic acid. However, the authors also indicated a possible contribution of other aroma compounds that were not commercially available at that time.

A comparison of the volatile compositions of full- and reduced-fat Cheddar showed that the level of methanethiol in the cheese is highly correlated with the flavor grade. This observation may indicate that the lack of aroma in reduced-fat Cheddar is due to lack of methanethiol. However, a combination of methanethiol and decanoic acid or butanoic acid in all cheeses gave a better correlation with Cheddar flavor than methanethiol alone (Dimos et al., 1996). Addition of methanethiol to bland slurry of reduced-fat Cheddar produced a strong Cheddar aroma (Urbach, 1997b).

Milo and Reineccius (1997) applied both traditional high vacuum isolation and AEDA and GCO of decreasing headspace samples (GCO-H) to further study the aroma of a regular and a low-fat Cheddar cheese (Table 12.4). After the quantification and calculation of respective odor activity values, based on sensory thresholds in oil and water, they suggested acetic acid, butyric acid, methional, 2,3-butanedione, and homofuraneol as the primary odorants responsible for the pleasant mild aroma of Cheddar cheese. In addition to the above-mentioned compounds, the contribution of highly volatile sulfur compounds such as methanethiol and dimethyl sulfide to nasal perception of Cheddar cheese was quite obvious on the basis of GCO analysis of static headspace samples. The authors further hypothesized that the meaty-brothy odor characteristic of low-fat Cheddar was caused by high concentrations of methional, HDMF, and especially homofuraneol. The HDMF-type odorants are known to be produced by certain strains of lactobacilli (Preininger, 1995 cited from Milo and Reineccius, 1997). While the mixture of these volatile compounds in a model cheese matrix had Cheddar aroma, attribute profilin described it as

lacking in sour, moldy, and sulfurous notes relative to the real cheese. Also, the overall odor was described as weak. This discrepancy in sensory character between the aromatized model and real cheese was partially caused by aroma-matrix interactions, which resulted in quantitative errors (Wang and Reineccius, 1998).

The use of DHDA methodology has suggested additional volatiles as being important to Cheddar cheese aroma as compared to the aforementioned results from GCO-H and solvent extraction/AEDA (Zehentbauer and Reineccius, 2002; Table 12.4). Results of DHDA showed that in addition to the odorants previously identified by AEDA and GCO-H, (*Z*)-4-heptenal, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, (*Z*)-1,5-octadiene-3-one, and (*E*)/(*Z*)-2-nonenal, which have been underestimated or not even perceived during AEDA, may also contribute to the overall aroma of Cheddar cheese.

The volatile aroma components of two sharp Cheddar cheeses of British Farmhouse origin, made using raw milk and ripened for at least 1 year, were analyzed by AEDA (Suriyaphan et al., 2001b; Table 12.4).

Descriptive sensory analysis of these cheeses was also conducted. Key flavors in sharp Cheddar cheeses were "barnyard" and "earthy." Following instrumental analysis, model system addition was used to confirm compounds responsible for specific flavor notes. *p*-Cresol was mainly responsible for a "cowy-barny" note, whereas an intense "soil-like" note was due to 2-isopropyl-3-methoxypyrazine. At much lower odor intensity, 2-isobutyl-3-methoxypyrazine contributed a "bell pepper-like" note. Direct addition of *p*-cresol (>100 ppb) or 2-isopropyl-3-methoxypyrazine (>3 ppb) in a mild domestic Cheddar cheese resulted in increases in intensities of cowy/phenolic and earthy/bell pepper aroma notes. Additionally, within the same wedge of cheese, the concentrations of *p*-cresol and 2-isopropyl-3-methoxypyrazine were lower at the center than at the rind.

Avsar et al. (2004) determined that the Strecker aldehydes 2/3-methyl butanal and 2-methyl propanal, which are derived from leucine, isoleucine, and valine, have central roles in the nutty flavor of Cheddar cheese. Nutty flavors are desirable, but they are generally only found in extremely aged Cheddar cheeses (>9 months; Avsar et al., 2004). After the identification of volatiles responsible for nutty flavor in Cheddar, Carunchia-Whetstine et al. (2006) attempted to create this specific aged cheese flavor note by employing specific culture of adjunct in pilot scale cheese making trials. Adding *L. lactis* ATCC

Table 12.4. Aroma Impact Compounds Identified in Cheddar and Hard Italian Cheeses

Mild Cheddar ^a	Sharp Cheddar (British Farmhouse) ^b	Grana Padano ^c	Parmigiano Reggiano ^d
HDMF	2-Isopropyl-3-methoxypyrazine	Ethyl hexanoate	3-Methylbutanal
(<i>E</i>)-2-Nonenal	3-(Methylthio) propanal	Ethyl butanoate	2-Methylpropanal
2,3-Butanedione	<i>p</i> -Cresol	2-Heptanol	2-Methylbutanal
(<i>Z</i>)-4-Heptenal	δ-Dodecalactone	1-Octen-3-ol	Dimethyl trisulfid
3-(Methylthio) propanal	Butanoic acid	3-(Methylthio) propanal	2,3-Butanedione
1-Octen-3-one	3-methyl butanoic acid	Nonanal	3-(Methylthio) propanal
2-Acetyl-2-thiazoline	2-Phenylethanol	Butanoic acid	Phenyl acetaldehyde
Dimethyl trisulfid	Ethyl octanoate	Hexanoic acid	Ethyl butanoate
(<i>Z</i>)-1,5-Octadien-3-one	Acetic acid		Ethyl hexanoate
(<i>Z</i>)-2-Nonenal	β-Damascenone		Ethyl octanoate
Ethyl butanoate	Octanoic acid		Acetic acid
Hexanal	Sotolon		Butanoic acid
2-Isobutyl-3-methoxypyrazine	Phenyl acetic acid		Hexanoic acid
<i>trans</i> -4,5-Epoxy-2-(<i>E</i>)-decenal	Ethyl butanoate		Octanoic acid
2-Nonanone	Ethyl hexanoate		
2-Isopropyl-3-methoxypyrazine	Dimethyl trisulfid		
Decanal	Phenyl acetaldehyde		
2/3-Methyl butanal	Pentanoic acid		
Ethyl octanoate	Guaiacol		
1-Hexen-3-one	γ-Decalactone		
Methyl propanal	δ-Decalactone		
Ethyl hexanoate	1-Octen-3-one		
Homofuraneol	2-Acetylpyrazine		
Butanoic acid	2-Isobutyl-3-methoxypyrazine		
	Linalool		
	(<i>E</i> , <i>Z</i>)-2,6-Nonadienal		
	Geosmin		
	HDMF		

^a Mild Cheddar cheese analyzed by GCO/AEDA/GC-MS and GCO-DHDA/GC-MS (Zehentbauer and Reineccius, 2002).

^b British farmhouse Cheddar cheese analyzed by GCO/AEDA/GC-MS (Suriyaphan et al., 2001b).

^c Grana Padano cheese analyzed by GCO/CHARM/GC-MS (Moio and Addeo, 1998).

^d Parmigiano Reggiano cheese analyzed by DH-GC-MS and OAV (Qian and Reineccius, 2002).

Table 12.5. Specific Flavor and Off-Flavor Compounds in Cheeses

Defect	Chemicals	Mechanism of Formation	Reference
Feta			
Kerosene-like	<i>trans</i> -1,3-Pentadiene	Microbial, enzymatic	Horwood et al. (1981a)
Goat cheese			
Waxy/crayon	4-Methyl octanoic acid, 4-ethyl octanoic acid	Milk, enzymatic	Carunchia-Whetstine et al. (2003)
Oxidized	1-Heptanol, heptanal, nonanal, 2-decenal	Lipid oxidation, light-induced	Kim et al. (2003)
Smear-coated cheese			
Potato-like aroma	2-Methoxy-3-isopropylpyrazine	Microbial	Dumont et al. (1983)
Brie/Camembert			
Musty/earthy	Methylisoborneol	Microbial	Karahadian et al. (1985)
Rotten soil/potato-like	3-Isopropyl-2-methoxypyrazine	Microbial	Curioni and Bosset (2002)
Gruyere de Comte			
Potato-like aroma	3-Methoxy-2-propylpyridine	Microbial	Dumont et al. (1975)
Swiss Gruyere			
Potato-like aroma	Methional	Microbial, enzymatic	Rychlik and Bosset (2001a,b)
Gouda			
Chemical (phenolic)	2-Bromo-4-methylphenol	Microbial/chemical	Mills et al. (1997)
Cheddar cheese			
Unclean-off-fl vor	2-Methyl propanoic acid, 3-methyl butanoic acid	Microbial, enzymatic	Nakae and Elliot (1965a,b)
Catty fl vour	2-Mercapto-2-methylpentan-4-one	Reaction of mesityl oxide (contaminant), and sulfid	Badings (1967); Spencer (1969a,b); Drake et al. (2001, 2002)
Fruitiness	Ethyl butanoate, ethyl hexanoate, ethyl octanoate	Microbial, enzymatic	Bills et al. (1965); Morgan (1970)
Floral/rose-like	Phenyl ethanol, Phenyl acetaldehyde	Microbial, enzymatic	Dunn and Lindsay (1985)

Unclean, utensil-like	<i>p</i> -Cresol(Off-fl vor enhanced by FFAs)	Microbial, enzymatic	Dunn and Lindsay (1985)
Unclean (dull harsh)	2/3-Methylbutanal, 2-methylpropanal	Microbial, enzymatic, Strecker degradation	Dunn and Lindsay (1985)
Rosy/flora	Phenylacetaldehyde, phenylacetic acid	Microbial, enzymatic	Carunchia-Whetstine et al. (2005)
Yeasty fl vor	Ethanol, ethyl acetate, ethyl butanoate	Microbial, enzymatic	Horwood et al. (1987)
Phenolic	<i>p</i> -Cresol	Microbial, enzymatic	Ramshaw et al. (1990)
Mayonnaise/bread-like	(<i>E, E</i>)/(<i>E, Z</i>)-2,4-Decadienal	Microbial, lipid oxidation, enzymatic	Suriyaphan et al. (1999, 2001a)
Cow/barny-fl vors	<i>p</i> -Cresol	Microbial, enzymatic	Suriyaphan et al. (2001b)
Earthy/bell pepper	2-isopropyl-3-methoxypyrazine	Microbial, Maillard reaction	Suriyaphan et al. (2001b)
Brothy f vor	3-(Methylthio) propanal, HDMF, Ethyl furaneol, 2-Methyl-3-furanthiol (and its dimerized form)	Microbial, enzymatic, Maillard reaction	Singh et al. (2004a); Cadwallader et al. (2006)
Nutty fl vor	2-Methylpropanal, 2/3-Methylbutanal	Microbial, enzymatic, Strecker degradation	Avsar et al. (2004); Carunchia-Whetstine et al. (2006)
Mothball/grassy	Acetic acid, 2-methyl butanoic acid, skatole	Microbial, enzymatic	Drake et al. (2007b)
Italian cheeses (Provolone, Pecorino, Romano, and Parmesan)			
Medicinal/cow	Phenol, <i>m</i> - <i>p</i> -cresol, ethyl phenol, 3,4-dimethyl phenol, 2-isopropyl phenol, thymol and carvacrol	Microbial, enzymatic, feed	Ha and Linsay (1991); Ney (1973)
Club cheese			
Musty/dirty	2-Alkanone (C7,9,11), 8-nonen-2-one	Mold growth/metabolites	Marsili (2007b)

Modified from Singh et al. (2007).

29146 at the rate of 10^4 or 10^5 CFU/mL of milk as an adjunct culture during manufacture of Cheddar cheese resulted in an increase in nutty fl vor perception in the cheese. Cheeses ripened at 13°C developed aged fl vors (including nutty) more rapidly than cheeses ripened at 5°C . Panelists described the 1-week-old cheeses as nutty/malty, whereas the 4- and 8-month cheeses were only described as nutty. The concentrations of 2/3-methyl butanal and 2-methyl propanal increased during aging and were higher in the cheeses with the adjunct culture added and in the cheeses ripened at 13°C . This study demonstrates the advantages of linking descriptive sensory analysis, fl vor chemistry, and starter culture biochemistry to control cheese fl vor. These results allow cheese manufacturers the opportunity to optimize the cheese-making procedure to produce a consistent nutty-fl vored Cheddar cheese.

It is important to note that in each of the studies mentioned previously, different Cheddar cheeses of different ages, microflora and biochemistry were studied. Cheddar cheese encompasses a wide category and there are numerous potential fl vor profiles. Thus, to elucidate Cheddar cheese fl vor is a large task and descriptive sensory analysis should be conducted in conjunction with any instrumental study to provide clarification.

Aroma Impact Compounds of Dutch Cheeses

The biochemical changes in Gouda cheese during manufacture and ripening is extensively researched. In the last decade, a number of studies on the fl vor chemistry were also published but surprisingly detailed understanding on the key odorants in Gouda cheese has still not been elucidated by sensory directed fl vor analysis. Engels (1997) outlined number of potent odorants in Gouda, this included compounds originating from metabolism of amino acids (3-methyl butanal, 3-methyl butanol, methanethiol, dimethyl sulfide/trisulfid 2-methyl propanal), sugar (diacetyl), lipid (butanoic acid, hexanal, pentanal), and combined pathways (ethyl butyrate).

Aroma Impact Compounds of Hard Italian Cheeses

Grana Padano (GP) cheese is an Italian hard cheese with a delicate and characteristic aroma, made from raw bovine milk, partly skimmed by creaming. These types of cheeses are manufactured with the addition of natural starter, cooked at 53°C , and ripened for at

least 1 year (Moio and Addeo, 1998). Fruity notes, which characterized fl vor of fresh GP, seems to be partly lost during the ripening process, while more complex aroma composed of roasted nut, peanut butter, baked potato, and earthy odor developed. Key odorants identified in 1-year-old GP cheese made up of esters, amino acid, and lipid degradation products (Table 12.4; Moio and Addeo, 1998).

The fl vor profile of Parmigiano-Reggiano cheese was dominated by malty, sulfury, floral/fruit, and cheesy/sweaty notes. Qian and Reineccius (2002) determined key aroma compounds of Parmigiano-Reggiano cheese by dynamic headspace GCMS and odor activity value (Table 12.4).

SPECIFIC FLAVORS OR OFF-FLAVORS IN CHEESES

In addition to the characteristic desirable fl vors, cheeses frequently suffer from specific fl vor defects. While desirable fl vor has been difficult to define in chemical and sensory terms since consumers vary in preference and definition of dairy products fl vor, the specific cause(s) of many of these specific fl vor or off-fl vor notes have been established more or less definitively. The volatile chemical(s) responsible for the specific fl vor/off-fl vor notes in cheeses are summarized in Table 12.5.

Specific fl vors like nutty or brothy favors were also characterized in Cheddar cheese (Table 12.5), which may or may not be considered as an off-fl vor depending on the consumer preference.

TASTE COMPOUNDS IN CHEESE

Research on taste compounds in dairy products is fairly limited but there seems to be an increasing interest in this area in recent years. In food systems such as milk and milk products, study of taste sensation/compounds in isolation or devoid of contribution from volatile aroma compounds is difficult due to complex nature, in terms of both number of food constituents and their competing/synergistic effect on taste and/or aroma. Compounds which contribute to taste of cheese are summarized in Table 12.6.

Important details available in the literature on taste compounds are summarized below:

- Sodium chloride is an important contributor to the taste of cheeses. The apparent saltiness of cheese increases with maturity, increased NaCl concentration, and decreasing pH (McSweeney, 1997).

Table 12.6. Taste Compounds in Cheese

Compounds	Taste/Other Complex Sensation	Products
Lactose/Glu/Gal	Sweet	Milk, cheese curd, unripened/fresh cheeses
Lactic acid	Sour	Fermented milks/cream, cheeses
Acetic/propanoic acid	Sour	Fermented milks, cheeses
Propionic acid	Sour, umami	Umami taste in Swiss cheese
Succinic acid	Umami	Umami taste in Swiss cheese
Ca/Mg salt of propanoic acid	Sweet	Cheeses
Peptides	Mostly bland, some can be bitter, sour or umami	Cheeses, fermented milks
γ -Glutamyl dipeptides	Sour, salty, brothy metallic	Comte cheese
<i>Amino acids</i>		
Gly, Ala, Ser, Thr	Sweet	Cheeses
Glu, Asp, Gln, Asn	Sour, umami	Cheeses
His	Sour (?)	Cheeses
Pro, Lys	Sweet, bitter	
Leu, Val, Ile, Arg, Phe, Tyr	Bitter	Cheeses
Trp		
NaCl	Salty	Cheeses
Ethanol	Slightly sweet, cooling/drying sensation	Cheeses, fermented milks

Modified from Singh et al. (2007).

- The principal acid in cheese is lactic acid. The concentration of lactic acid, and also the pH, varies considerably with:
 - the type of fermented dairy products
 - initial production by the starter culture
 - extent of loss in whey
 - its metabolism by the nonstarter microflora

Several other acids, for example, acetic, propanoic, and C_{4-10} , also contribute to sour/soapy taste but they mostly contribute toward the aroma. Some of the characteristic taste (sour, sweet, salty) compounds of Emmentaler (Swiss) cheese were acetic acid, propanoic acid, lactic acid, succinic acid, and glutamic acid, each in free form and/or as ammonium, sodium, potassium, magnesium, and calcium salts as well as corresponding chlorides and phosphates (Warmke et al., 1996). Magnesium and calcium propionate mainly caused the sweetish note in the taste margin of Emmentaler cheese:

- Casein is hydrolyzed to varying degrees depending on the type of fermented milk and cheese, resulting in the production of peptides and free amino acids. The precise role of the intermediate to small molecular weight peptides is not clear; however, it is generally accepted that it plays an important role in the background taste of cheese (Fox et al., 1994). Several peptides were identified in different types of cheeses as bitter (see Table 12.7 for a list of bitter peptides).
- An interesting relationship was established by Ney (1981) between the average hydrophobicity (Q) of a peptide, as measured by the hydrophobicity of amino acid side chains determined by Tanford (1962), and bitterness. The peptides with Q values >1400 cal/mol/residue and molecular weights up to 6000 Da (molecules >6000 Da are likely to be too large to interact with the taste receptors) taste bitter, and no bitterness occurs when Q is <1300 .

Table 12.7. Bitter Peptides Identified in Cheeses

Peptide	Sequence	Hydrophobicity (cal/mol/residue)	Type of Cheese	Reference
α_{s1} -CN f1-7	H.Arg.Pro.Lys.His. Pro.Ile.Lys.OH	1771.0	Cheddar	Lee et al. (1996)
α_{s1} -CN f1-13	H.Arg.Pro.Lys.His. Pro.Ile.Lys.His. Gln.Gly.Leu.Pro.Gln.OH	1363.0	Cheddar	Lee et al. (1996)
α_{s1} -CN f11-14	H.Leu.Pro.Gln.Glu.OH	1367.0	Cheddar	Lee et al. (1996)
α_{s1} -CN f14-17	H.Glu.Val.Leu.Asn.OH	1162.5		Hodges et al. (1972); Richardson and Creamer (1973); Hamilton et al. (1974)
α_{s1} -CN f17-21	H.Asn.Glu.Asn. Leu.Leu.OH	1074.0	Cheddar	Hodges et al. (1972); Richardson and Creamer (1973); Hamilton et al. (1974)
α_{s1} -CN f26-32	H.Ala.Pro.Phe. Pro.Glu.Val.Phe.OH	1930.0	Cheddar	Richardson and Creamer (1973)
α_{s1} -CN f26-33	H.Ala.Pro.Phe. Pro.Glu.Val.Phe.Gly.OH	1688.8	Cheddar	Hodges et al. (1972); Hamilton et al. (1974)
α_{s1} -CN f198-199	H.Leu.Trp.OH	2710.0	Alpkäse	Guigoz and Solms (1974)
α_{s2} -CN f191-197	H.Lys.Pro.Trp. Ile.Gln.Pro.Lys.OH	2010.0	Cheddar	Lee et al. (1996)
β -CN f8-16	H.Val.Pro.Gly.Glu.Ile. Val.Glu.Ser(P).Leu.OH	1390.0	Cheddar	Lee et al. (1996)
β -CN f 46-67	H.Gln.Asp.Lys.Ile. His.Pro.Phe.Ala. Gln.Thr.Gln.Ser. Leu.Val.Tyr.Pro. Phe.Pro.Gly. Pro.Ile.(Pro/His).OH	1580.5	Cheddar	Richardson and Creamer (1973); Hamilton et al. (1974)
β -CN f 61-69	H.Pro.Phe.Pro.Gly. Pro.Ile.Pro.Asn.Ser.OH	1792.2	Butterkäse	Huber and Klostermeyer (1974)
β -CN f46-84	H.Gln.Asp.Lys. Ile.His.Pro.Phe. Ala.Gln.Thr.Gln.Ser. Leu.Val.Tyr.Pro.Phe. Pro.Gly.Pro.Ile. (Pro/His).Asn.Ser.Leu. Pro.Gln.Asn.Ile.Pro. Pro.Leu.Thr.Gln.Thr. Pro.Val. Val.Val.OH	1508.5	Cheddar	Hamilton et al. (1974)
β -CN f84-89	H.Val.Pro.Pro. Phe.Leu.Gln.OH	1983.3	Gouda	Visser et al. (1983)
β -CN f193-209	H.Tyr.Gln.Glu. Pro.Val.Leu.Gly.Pro. Val.Arg.Gly.Pro. Phe.Pro.Ile. Ile.Val.OH	1762.4	Cheddar, Gouda	Kelly (1993); Broadbent et al. (1998); Soeryapranata et al. (2002a, b); Singh et al. (2004b, 2005); Visser et al. (1983)
β -CN f193-208	H.Tyr.Gln.Glu. Pro.Val.Leu.Gly. Pro.Val.Arg.Gly. Pro.Phe.Pro.Ile.Ile.OH	1766.9	Gouda	Visser et al. (1983)
β -CN f193-207	H.Tyr.Gln.Glu. Pro.Val.Leu.Gly. Pro.Val.Arg.Gly. Pro.Phe.Pro.Ile.OH	1686.7	Gouda	Visser et al. (1983)

Modified from Singh et al. (2007).

cal/mol/residue. Peptide β -CN f193-209, identified in both Cheddar and Gouda, determined to be bitter by detailed sensory analysis (Singh et al., 2004b). This peptide with Q value 1839 cal/mol/residue and molecular weight 1882.51 Da was also classified as potentially bitter in the Q value model proposed by Ney (1981). Amino acids are also known to elicit different taste (see Table 12.6).

- Several small peptides were identified in Comté cheese. Cyclic dipeptides were described as bitter (Roudot-Algaron et al., 1993) and dipeptides with a gamma-glutamyl residue were found sour (e.g., γ -Glu-Tyr) apart from γ -Glu-Phe described with a complex taste, which was brothy and slightly sour, salty, and metallic (Roudot-Algaron et al., 1994). However, because of their low concentration compared to their taste threshold value they could not be directly responsible for cheese taste.
- Seven low molecular weight peptides, namely H.Leu.Pro.OH, H.Val.Pro.OH, H.Phe.Pro.OH, H.Lys.Pro.OH, H.Gly.Pro.Val.Arg.OH, H.Tyr.Pro.OH, and H.Arg.Pro.OH, were identified in Vacherin Mont d'Or cheese (Mojarro-Guerra et al., 1991). Most of these peptides (H.Lys.Pro.OH, H.Phe.Pro.OH, H.Val.Pro.OH, and H.Leu.Pro.OH) were bitter. The cheese extract was evaluated as "brothy, sour, and bitter," and the authors concluded that the bitter-tasting peptides identified contributed in a positive way to overall flavor.

Recently, a number of workers studied taste active compounds in Camembert (Engel et al., 2001a,b,c), Cheddar (Yang and Vickers, 2004), Comté (Salles et al., 1995), Goat (Engel et al., 2000a,b; Engel et al., 2002; Salles et al., 2002), and Emmentaler cheeses (Warmke et al., 1996).

In a recently published study by Drake et al. (2007c) studied compounds responsible for the umami taste in Cheddar and Swiss cheeses. Low- and high-intensity umami tasting cheeses were selected using trained sensory panel. Some compounds, namely monosodium glutamate (MSG), disodium-5'-inosine monophosphate (IMP), disodium-5'-guanosine monophosphate (GMP), sodium chloride, lactic acid, propionic acid, and succinic acid, were quantified in both types of cheese with and without umami taste. Comparison of analytical data and sensory thresholds indicated that IMP and GMP thresholds were 100-fold higher than their concentrations

in cheese. All other compounds contributed some umami taste within their concentration range in umami cheeses. Sensory analysis of model cheeses clearly demonstrated that Glu played a major role in umami taste of both Cheddar and Swiss cheese while succinic and propionic acids contributed to Swiss cheese. The knowledge of umami tasting components of cheeses will be useful in developing technologies to control and regulate the level of this specific taste attribute in cheeses.

CONCLUSIONS

A concerted series of chemical and biochemical reactions are involved in the formation of cheese flavor and off-flavor compounds. The general chemical/biochemical pathways, that is (1) glycolysis, (2) lipolysis, and (3) proteolysis, involved in the degradation of milk constituents are now fairly well characterized. Recent works on the enzymology and genetic manipulation of the starter and nonstarter lactic acid bacteria have helped in better understanding of further catabolic modification of the products of primary degradation pathways. This has led to immense progress in understanding of the cheese flavor chemistry. So far a large number of volatile compounds have been identified from various types of cheeses but still it is not possible to duplicate cheese flavor by pure chemicals in model systems. However, there is now good understanding on the causes of bitterness and specific flavors/off-flavors in dairy products.

Recent developments in sensory and instrumental methodologies in flavor analysis have been of immense help in furthering our understanding of the cheese flavor chemistry. Further work on the characterization of flavor (both aroma- and taste-active) compounds, flavor-matrix interaction mechanisms and flavor release mechanisms are needed to fully elucidate the complex nature of cheese flavors. The better understanding of flavor chemistry will be useful in the development of new technologies/mechanisms for the effective control and acceleration of the ripening process in cheese.

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13

Evaporated and Sweetened Condensed Milks

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HISTORICAL PERSPECTIVE AND PRODUCT IDENTITY

The process to evaporate and preserve milk in a sealed container was first introduced in France by Nicolas Appert. He began his experiments in 1795 when France, under Napoleon, was fighting with other European nations. The need for transportable food was one of the greatest problems for the French army at that time. Hence, the French government offered a

prize of 12,000 francs—which was a huge sum in those days—to anyone who could find a satisfactory method of preserving food. After 15 years of experimentation, Nicolas Appert announced that he could keep foods for a long period by cooking the food, sealing it in an airtight container, and cooking it again. On January 30, 1810, Appert was awarded the prize which earned him the title, the “Father of the Canning Industry.” This was the beginning of the art of canning. Appert’s process involved boiling milk in an open kettle to one-third of its original volume. He then put the milk in bottles, tightly corked the bottles, and then heated the bottle and its contents again in a hot water bath. The reason why food cooked and sealed in an air-tight container remained in a good condition was not explained until another Frenchman, Louis Pasteur demonstrated the nature and the behavior of microscopic organisms and solved the riddle.

In 1813, an English patent awarded to Edward Howard described a “vacuum pan” in which milk boiled vigorously at a low temperature of 54.4°C (130°F) and half of the water removed. In England, a vacuum pan was used for the first time in connection with evaporation of milk in 1835. Being struck by the suffering of infants on a prolonged Atlantic trip, Gail Borden researched for several years to develop concentrated milk that can be marketed. In 1856, Gail Borden received his first patent in the United States and England for preserving milk in a semifluid state after evaporation in vacuum. This was the birth of the first sweetened condensed milk (SCM) in hermetically sealed cans. Although the claim for Borden’s patent was for “producing concentrated sweet milk by evaporation in vacuo without the admixture of sugar or other foreign matter,” his commercial

Table 13.1. Codex Standards for Different Evaporated Milks

	Evaporated Milk	Evaporated Skimmed Milk	Evaporated Partly Skimmed Milk	Evaporated High-Fat Milk
Minimum milk fat (%)	7.5	1	>1 but <7.5	15
Minimum milk solids ^a (%)	25	20	20	11.5
Minimum milk protein in milk solids-not-fat ^a (%)	34	34	34	34

^a Milk solids and milk solids-not-fat content include water of crystallization of lactose.

development was in the manufacture of SCM. In 1857, an English patent was granted to Joseph House for preserving unsweetened condensed milk.

However, the first reported commercial success of SCM was the establishment in 1866 at Charn, Switzerland, of the Anglo-Swiss Condensed Milk Company by three American brothers named Paige. John B. Meyenberg—a Swiss who worked for Anglo-Swiss Company was convinced that evaporated milk could be preserved without sweetening. His employers rejected the idea prompting Meyenberg to resign and relocate to the United States. In 1884, Meyenberg received a U.S. patent for the process of sterilization by steam while cans were agitated. The Helvetia Milk Condensing Company in Highland, Illinois, was born—producing the first commercial evaporated milk in 1885. In 1901, machine-made evaporated milk cans were introduced to replace laboratory-fabricated cans. Meanwhile, in 1904, the Anglo-Swiss Company merged with Henry Nestlé of Vevey, Switzerland, to form the Nestlé-Anglo-Swiss Condensed Milk Company.

The process of homogenization during the production of evaporated milk was introduced in 1909 to stabilize the emulsion and to reduce fat separation. In 1922, the continuous system for sterilizing evaporated milk was developed and in 1923, the U.S. Department of Agriculture (USDA) promulgated an advisory standard for condensed milk, evaporated milk, and concentrated milk. For more on the historical developments and perspectives, refer to Parfit (1956) and Bell (1962).

TYPES OF EVAPORATED AND SWEETENED CONDENSED MILKS

Unsweetened condensed milk and SCM can be made from fresh milk or from recombined milk [nonfat dry milk (NFD), fat, and water]. When the source of

fat is other than butterfat, the resultant milk is called *fat milk*. The US Code of Federal Regulations (CFR) (Code of Federal Regulations, 2007) define concentrated, evaporated, and sweetened condensed milks as follows:

Concentrated milk, also called condensed milk, by definition is product obtained by partial removal of water from milk. It contains not less than 7.5% milk fat and not less than 25.5% total milk solids. It is pasteurized but not processed by heat to prevent spoilage and it may be homogenized. Vitamin addition is optional. If added, the quantity of Vitamin D in each fluid ounce (~ 0.03 L) is 25 I.U. (21CFR131.115).

Evaporated milk, also called “unsweetened evaporated milk,” is about 2× concentrated whole milk. It is made by removing about 60% of the water in milk. It contains not less than 6.5% milk fat, not less than 16.5% milk solids-not-fat (SNF) and not less than 23% by weight of total milk solids. It contains added vitamin D (25 IU per fluid ounce). It is heat sterilized to prevent spoilage. Optional ingredients may include vitamin A (125 IU per fluid ounce), stabilizers and emulsifier (21CFR131.130).

International standards (CODEX STAN A-3-1971, Rev. 1-1999) (Codex Alimentarius, 2007a,b) specify four types of evaporated milks (Table 13.1).

Condensed milk may be produced from milk and milk powders, cream and cream powders, and milk fat products. The protein content may be adjusted with lactose or with retentate or permeate obtained from ultrafiltration of milk, partly skim milk, or skim milk. Permitted ingredients are potable water, sugar, and sodium chloride. Allowed food additives are firming agents (potassium or calcium chloride—used at rate of 2 g/kg singly or 3 g/kg in combination, expressed as anhydrous substances), stabilizers (sodium, potassium, or calcium citrates—used at rate of 2 g/kg singly or 3 g/kg in combination, expressed as anhydrous substances), or acidifier (carbonates and phosphates of sodium, potassium, or calcium; di-, tri-, and

Table 13.2. Codex Standards for Different Sweetened Condensed Milks

	Sweetened Condensed Milk	Sweetened Condensed Skim Milk	Sweetened Condensed Partly Skimmed Milk	Sweetened Condensed High-Fat Milk
Fat	8	1	>1 to <8	16
Milk solids-not-fat	Not specific	Not specific	20	14
Milk solids ^a	28	24	24	
Milk protein in milk solids-not-fat ^a	34	34	34	34

^a Milk solids and milk solids-not-fat content include water of crystallization of lactose.

polyphosphates—used at rate of 2 g/kg singly or 3 g/kg in combination, expressed as anhydrous substances); carrageenan (150 mg/kg) as thickener and lecithin as emulsifier used at levels permitted by good manufacturing practices.

SCM, also called “condensed milk,” is concentrated milk solids made by removal of about 60% water from a mixture of milk (whole, nonfat, or homogenized milk) and safe and suitable nutritive carbohydrate such as sucrose. It contains not less than 8% milk fat and not less than 28% total milk solids. The product is pasteurized and may be homogenized (21CFR131.120). International standards for SCM require a minimum fat content of 8% and a minimum of 28% total milk solids. The minimum sugar content is not specific but is usually above 40% and should be sufficient to prevent spoilage. Addition of fruit juices or concentrates, coloring, and natural and artificial flavors are permitted.

CODEX STAN A-4-1971, Rev 1-1999 allows the use of the following raw materials in SCM: milk, milk powders, cream and cream powders, and milk fat products. Other dairy products used as raw material for protein adjustment are milk retentate, permeate, and lactose (also for seeding). The products are in such a way as not to alter the whey protein to casein ratio in the milk being adjusted. Permitted ingredients are potable water, sugar, and sodium chloride. According to the CODEX standards, the finished food contains not less than 8% by weight of milk fat and not less than 28% by weight of total milk solids, and protein not less than 34% of the SNF. Allowed food additives are firming agents (potassium or calcium chloride—used at rate of 2 g/kg singly or 3 g/kg in combination), stabilizers (sodium, potassium, or calcium citrates—used at rate of 2 g/kg singly or 3 g/kg in combination), or acidifier (carbonates and phosphates of sodium, potassium, or calcium; di-, tri-, and polyphosphates—used at rate of

2 g/kg singly or 3 g/kg in combination); carrageenan (150 mg/kg) as thickener and lecithin as emulsifier used at levels permitted by good manufacturing practices. Codex standards allow for four types of SCM (Table 13.2).

The average compositions of evaporated and sweetened condensed milk compared to whole milk are given in Table 13.3.

PRODUCTION OF EVAPORATED AND SWEETENED CONDENSED MILKS

The production of condensed, concentrated, and evaporated milks (sweetened or unsweetened) in the United States has remained essentially flat over the years—with most of the production being bulk condensed skim milk (USDA, 2007) (Table 13.4). Condensed milk production is also high in European countries. The Netherlands is one of the major players in condensed milk products worldwide—holding a 20% share in global trade volume. In 2006, nearly 309,000 tons (~618 million pounds) of condensed milk was produced in the Netherlands (Dutch Agriculture Statistics, 2007). In the UK, ~303 million liters (~689 million pounds) of condensed milk was produced in 2006 (UK Agriculture Statistics, 2007).

TECHNOLOGIES FOR MANUFACTURE OF CONDENSED AND EVAPORATED MILKS

Evaporation is used to concentrate milk before drying or transportation. If milk is to be transported long distances, it is concentrated to 30–38% total solids to reduce transportation costs. Evaporation is thought to be one of the most energy-intensive processes in the dairy industry. Hence, modern evaporators are

Table 13.3. Proximate Composition of Whole, Evaporated, and Sweetened Condensed Milks

Component	Whole Milk	Evaporated Milk	Sweetened Condensed Milk
Protein (%)	3.2	7.0	8.2
Moisture (%)	88	74	26
Fat (%)	3.5	7.5	8.0
Carbohydrates (%)	4.6	9.6	55.1
Lactose (%)	4.6	9.8	10–12
Sucrose (%)			44–46
Ash (%)	0.7	1.5	1.8
Calcium (mg)	120	228	238
Phosphorus (mg)	90	213	236
Sodium (mg)	53	94	88
Potassium (mg)	136	297	360

efficiently designed for low energy consumption and also to provide minimal heat damage to milk components.

There are basically two types of evaporator designs—tubular and plate—that may be single effect or multiple effects of two or more units. Listed below are various commercial types of evaporators.

- Falling film evaporators
- Rising film evaporators
- Forced circulation evaporators
- Plate evaporators
- Vapor recompression evaporators

FALLING FILM EVAPORATORS

The falling film tubular evaporator is primarily used in the dairy industry. In the falling film evaporators, liquid and vapors flow downward in parallel flow. The liquid to be concentrated is preheated to boiling temperature. An even thin film enters the heating tubes

via a distribution device in the head of the evaporator, flows downward at boiling temperature, and is partially evaporated. This gravity-induced downward movement is increasingly augmented by the cocurrent vapor flow.

Falling film evaporators can be operated with very low temperature differences between the heating media and the boiling liquid, and they also have very short product contact times, typically just a few seconds per pass. These characteristics make the falling film evaporator particularly suitable for heat-sensitive products such as milk.

RIISING FILM EVAPORATORS

In the rising film evaporator, feed enters the bottom of the heating tubes and as it heats, steam begins to form. The ascending force of this steam produced during the boiling causes liquid and vapors to flow upward in parallel flow. At the same time, the production of vapor increases and the product is pressed as a thin film

Table 13.4. Production Condensed and Canned Milk in the United States (2001–2005)

Year	Bulk Condensed Whole Milk	Bulk Condensed Skim Milk	Evaporated Skim Milk	Evaporated and Condensed Whole Milk
2001	140.3	937.0	15.0	452.8
2002	132.9	1,035.6	19.7	573.2
2003	204.3	919.0	17.5	577.8
2004	193.0	903.7	19.1	529.9
2005	179.2	1,056.8	20.4	527.0

on the walls of the tubes, and the liquid rises upward. The cocurrent upward movement helps to create a high degree of turbulence in the liquid, making the process advantageous for evaporation of highly viscous products and products that have a tendency to foul the heating surfaces.

Falling film evaporators are often used with product recirculation, where some of the formed concentrate is reintroduced back to the feed inlet in order to produce sufficient liquid inside the boiling tubes.

FORCED CIRCULATION EVAPORATORS

Forced circulation evaporators are used if boiling of the product on the heating surfaces is to be avoided due to the fouling characteristics of the product, or to avoid crystallization. Forced circulation evaporators are usually for viscous liquids, corrosive liquids, and liquors that cause fouling and scaling problems.

PLATE EVAPORATORS

Instead of tube bundles, framed plates can be used as heating surface. These plate assemblies are similar to plate heat exchangers but are equipped with large passages for the vapor flow. In these units, a product plate and a steam plate are connected alternately. The product passage is designed for even distribution of liquid on the plate surfaces and low-pressure drop in the vapor phase.

VAPOR RECOMPRESSION EVAPORATORS

To minimize energy consumption, two systems for vapor recompression are in use. These are thermal vapor recompression (TVR) and mechanical vapor recompression (MVR) evaporators. In MVR evaporators, the heating medium in the first effect is vapor developed in the same effect, compressed to a higher temperature using turbocompressor or high-pressure fans (MVR). In TVR evaporators, the heating medium in the first effect is the product vapor from one of the next calandria, compressed to a higher temperature by steam injection. Vapor generated from each calandria is heating medium in the next, while vapor from the last effect is condensed and may be used as boiler or cleaning water or to preheat incoming air for spray drying. For further reading, see Hess (1993), Carić (1994), Walstra et al. (1999), and Niro Inc. (2007).

PROCESS FOR MANUFACTURE OF EVAPORATED MILK

The manufacture of evaporated milk involved two processes as follows:

1. Preparation (i.e., standardization, heat treatment, concentration/evaporation, homogenization, and cooling) of a concentrated milk
2. Canning and heat sterilization of concentrate
3. Cooling and storage

For good quality evaporated milk, incoming raw milk must be of good microbial and organoleptic quality. Limits for Grade A raw milk in the United States are microbial counts less than 100,000 per mL, coliform counts not exceeding 10 per mL, and somatic cell count of less than 750,000 per mL.

The raw milk is clarified, filtered, and standardized. For a stable product and to prevent coagulation during heat processing and minimize age thickening during storage, the milk is heat stabilized by adding small amounts of stabilizing agents like phosphates, citrates, and bicarbonates to maintain pH 6.6–6.7 during heat treatment. More commonly, both disodium orthophosphate (DSP) and monosodium orthophosphate (MSP) are used. DSP and MSP have opposite effects on the pH of the milk. Carrageenan is also added as a thickener. The heat stability of milk is influenced by several compositional factors including mineral (ash) content, protein, and acidity levels. Natural heat stability also varies seasonally and is influenced by stage of lactation.

PREHEATING

Preheating is done to reduce the microbial load, to inactivate some indigenous milk enzymes, and to enhance heat stability (i.e., increasing the resistance of the milk to coagulation during subsequent sterilization). In addition to the primary purpose of increasing heat stability, preheating also affects viscosity of the final product. Preheating is done in continuous heat exchangers (plate or tubular types) with long holding times. The time \times temperature requirements are usually $93\text{--}100^\circ\text{C} \times 10\text{--}25$ minutes or $115\text{--}128^\circ\text{C} \times 1\text{--}6$ minutes.

CONCENTRATION

Next, the heated milk is evaporated under vacuum (typically using any of the multiple effect evaporator types described earlier). Evaporation under vacuum

exposes milk to a pressure lower than atmospheric pressure and its boiling point is lowered to $\sim 45^{\circ}\text{C}$ (113°F). Typical evaporation temperatures used are not less than 45°C (seldom exceeding 54.5°C) in order to prevent growth of microorganisms. Also, evaporation under partial vacuum helps prevent undesirable changes to milk components (e.g., heat damage to milk proteins). The milk is concentrated to 30–40% solids. Although concentration by evaporation is the most commonly used method, the milk may also be concentrated by reverse osmosis. Reverse osmosis is a membrane filtration process used to remove water from milk, whey, etc., at lower energy costs compared to evaporation.

HOMOGENIZATION

The milk is then homogenized at high pressure, that is, 15–25 MPa (200–250 bar) in first stage and 5–10 MPa in second stage. The preferred homogenization temperature matches the evaporation temperature of more than 45°C . The purpose of homogenization is to prevent creaming and coalescence. Homogenization breaks down the fat globules from an average size of 3–5 μm or larger into smaller sizes of less than 1 μm resulting in improved color (natural white to light cream color) and stability of the milk. However, excessive homogenization pressure may result in irreversible destabilization effect and reduction in heat stability of the product. Following homogenization the product is cooled and placed into storage where the final standardization of composition takes place.

SECOND STANDARDIZATION AND STABILIZATION

During the second standardization and stabilization, the total solids content in the product is readjusted to meet required standards of the first standardization. The process of standardization can take several hours as the evaporated milk in storage tanks has to be analyzed prior to standardization. Water, skim milk, evaporated milk, retentate, permeate, and homogenized cream are often used for standardization of the ratio of fat to solids-not-fat. Also, the salt balance in the milk, hence pH, is adjusted with stabilizing salts to insure that the product withstands further intense heat treatment. Because the salts are added in aqueous form, the milk is concentrated to higher total solids content so that restandardization and stabilization is used to bring solids content to desired levels.

PACKAGING

The most common method for packaging evaporated milk is canning by means of a suitable filling machine with lid seaming. Typically, the can is manufactured with a lid that has a center hole (diameter 2–3 mm). When the can is filled with the product, filling must be done in a manner that does not allow foaming of the product. After the can is filled with the product through the center hole, it is immediately sealed hermetically (i.e., does not allow the passage of air or fluid in either direction) by soldering. Because the milk is cold before filling of can, sufficient headspace equal to $\sim 10\%$ of the can volume must be allowed for product expansion during sterilization. A large headspace may result in excessive foaming, clotting, and brown deposits around the corners of the can. Hence, the headspace can be partially evacuated by the injection of steam into the headspace of the can at the time of closing. A suitable vacuum level is 2–4 inches of Hg in the can after cooling.

STERILIZATION

The canned product is then sterilized in situ at $115\text{--}121^{\circ}\text{C}$ for 15–20 minutes followed by cooling for about 15 minutes to $25\text{--}30^{\circ}\text{C}$. This is called in-container sterilization. It can be done in batches or continuously. The purpose of sterilization is to kill bacterial spores and inactivate heat-stable indigenous milk enzymes such as plasmin and other bacterial enzymes that may be present in the milk. The adequacy of sterilization is checked by holding random samples of the cooled canned evaporated milk for 2–3 weeks at $27\text{--}30^{\circ}\text{C}$ and checking for spoilage (i.e., microbial growth, gas formation, bulging, and explosion of cans) before shipping. When product is to be shipped to warmer climates, incubation of the cans may be done at 37 or 55°C to detect facultative or obligate thermophilic bacteria, respectively.

For evaporated milk to have an acceptable shelf life at room temperature, it must be “commercially” sterilized. This means that it must not contain organisms that will grow under normal storage conditions. Although obligatory thermophilic organisms may grow at high temperatures such as 45°C , the product is still considered “commercially” sterile. A time–temperature combination to give “absolute” sterility is possible; however, the resultant product will have an unacceptable cooked flavor and a dark color resulting from Maillard browning.

Sterilization may also be done by ultra-high temperature (UHT) heating at 130–140°C using direct or indirect heating. After sterilization, the cans are sealed aseptically. Aseptic packaging occurs under pressure at temperatures exceeding 100°C.

SPOILAGE OF EVAPORATED MILK

Sterilization is designed to kill heat-resistant spore—most of which, in milk, are species of the genus *Bacillus* or occasionally *Clostridium*. However, inadequate cooling and/or storage at high temperatures may cause growth of some heat-resistant spores. The most heat-resistant spore in milk is *Bacillus stearothermophilus*. This organism may not grow in temperate climate but grows well under tropical conditions. *B. stearothermophilus* grows best at 37°C and above may cause acid coagulation and a slight cheesy odor. *Bacillus subtilis* causes nonacid curd with bitter taste. *Clostridium sp.* causes the putrefaction and gas production with a smell of H₂S.

MANUFACTURE OF SWEETENED CONDENSED MILK

SCM is manufactured from whole milk, skim milk, or recombined condensed milk (consisting of skim milk powder, anhydrous milk fat or vegetable fat, and water). The processing steps in the manufacture of SCM are as follows:

1. Standardization of milk
2. Heat treatment of milk
3. Evaporation
4. Adding sugar
5. Cooling
6. Seeding and subsequent cooling for crystallization
7. Canning and packaging

Raw milk of good microbiological quality and low spore counts is the preferred starting material. When NFDM (or skim milk powder) is used for recombined SCM, it must also have a good microbial quality and low spore counts.

The raw milk is standardized to 8.0% fat and 21% SNF giving Fat:SNF ratio of 0.381. Standardization is achieved using any of the ingredients described in the CODEX standard above or in respective country. Following standardization, the milk is given an initial heat treatment (80–120°C) similar to that used for evaporated milk manufacture. The heat treatment influence viscosity of the final product and age

thickening during storage. Heat treatment in the range of 90–100°C gives the product most susceptibility to age thickening during storage. In general, low heat treatments favor increased viscosity while high heat treatments give lower viscosity in SCM. Heating at 80–85°C for 15–25 minutes gives the desired initial viscosity with slow age thickening. Viscosity should be sufficient to prevent separation during storage and age thickening as the product should remain pourable during storage. The average viscosity of SCM is ~2 Pa-s, about 1,000 times the viscosity of milk. The heated milk is homogenized at low pressure (~2–6 MPa) because creaming is not often a problem in SCM.

The heated product is then condensed by evaporation under vacuum at 65–70°C in a multiple-effect evaporator. Reverse osmosis may also be used to concentrate the milk. Condensation temperatures lower than 65°C favor germination of spores and growth of heat-resistant bacteria. The concentrated product is then cooled to 20–30°C using vacuum coolers.

Because SCM is not heat sterilized, the addition of sugar serves to improve its keeping quality by providing a bacteriostatic environment. The preferred sugar used in SCM manufacture is sucrose (although glucose and other sugars have been used for diabetic products). The sucrose is added as crystalline form or as a solution by dissolving in water at about 95°C before adding to milk by high shear recirculation in the milk. The time for sugar addition influence the product quality. Adding sucrose before heat treatment increases heat resistance of bacteria and their enzymes leading to age thickening, and adding sucrose before evaporation also causes increased viscosity. Hence, the best time for adding sucrose to give optimal product quality is near the end of evaporation. The final concentration of sugar in the aqueous phase of SCM, known as “sugar number” or “sugar index,” is ~62.5–64.5%.

After addition of sugar, the fat and total solids content of the product is readjusted to desired levels to meet minimum standards.

LACTOSE SEEDING

Next, lactose crystallization is induced. Although the concentration of sugar in moisture is above 61%, osmophilic organisms can grow. Because part of the lactose contained in the product may be over saturated, autocrystallization of lactose may occur and lactose may appear as large crystals with size greater than 15 µm. The presence of large crystals results in a

defect known as “sandiness”—that is, a gritty mouthfeel when SCM is consumed. To avoid this defect, the concentrate is cooled to the optimal seeding temperature of $\sim 25\text{--}30^\circ\text{C}$ and inoculated or “seeded” with fin milled and pasteurized dry lactose crystals to promote instant and controlled crystallization. Below 20°C , lactose crystallizes instantly without seeding. At $30\text{--}50^\circ\text{C}$, less lactose crystallizes and above 50°C , lactose is in solution and does not crystallize. The amount of lactose added is equivalent to $0.5\text{ kg}/1,000\text{ kg milk}$. The smallest possible size for the seed lactose should be less than $10\text{ }\mu\text{m}$ with a significant portion less than $1.0\text{ }\mu\text{m}$. Rapid cooling and agitation occurs during seeding. The amount of crystals formed is more than $4 \times 10^{11}\text{ crystals}/\text{m}^3$. Lactose crystal size affects viscosity of SCM. During the first half of crystallization, the viscosity of SCM increases to a maximum because of small crystal size but as crystal size grows, viscosity decreases.

After crystallization is complete, the product is packaged by fill in metallic tin cans which have been previously sterilized by flaming. It is important that the air in the fill area is clean and filtered to avoid future microbial quality issues. Also, the fill can must have the lowest possible airspace (headspace) above the product to prevent mold growth.

DEFECTS IN SWEETENED CONDENSED MILK

MICROBIAL SPOILAGE

Because SCM is not a sterile product, it is prone to microbial spoilage although its low water activity (~ 0.83) and high sugar content prevent microbial growth. However, osmophilic yeasts of genus *Torulopsis* may grow to cause gas formation and bulging cans. They also cause coagulation and produce fruity flavors. Other microorganisms that grow in SCM are micrococci and molds such as *Aspergillus repens* and *Aspergillus glaucus* and *Penicillium* sp. which grow on the surface of SCM when sufficient air (and oxygen) is available.

CHEMICAL DEFECTS

The main chemical defect in SCM is age thickening followed by gelation. Factors that affect chemical defects include seasonal variation in milk composition, preheat treatment of milk, the stage at which

sugar is added, and the degree of concentration and stabilizing salts added. For example, early lactation milk is more sensitive to age gelation than mid-lactation milk; less age thickening occurs when milk is heated by UHT treatment with long heating times; late addition of sugar during the evaporation step reduces age thickening; and a high concentration factor increases age thickening. Age thickening is also influenced by the type, amount, and stage at which stabilizing salts are added. For example, adding 0.03% sodium tetra pyrophosphate (STP) delays age thickening whereas adding more STP promotes age thickening. Age thickening increases with storage temperature ($Q_{10} = \sim 3.4$) and in tropical climates, SCM gelation occurs in about 1 year. High-temperature storage may also lead to brown discoloration due to Maillard reaction.

RECOMBINED EVAPORATED AND SWEETENED CONDENSED MILK

In countries with limited milk supply, evaporated milk and SCM are manufactured using NFDM as starting material. Other ingredients used include sweet cream buttermilk powder, fat (anhydrous milk fat or vegetable fat), water, and stabilizing salts (Choat, 1979). When evaporated milk or SCM is manufactured using all dairy ingredients, the products are called recombined evaporated milk or recombined SCM, respectively. When vegetable fat is used instead of milk fat, the products are described as “filled milk (Fotheringham and Choat, 1979). The specific requirement of the NFDM used for manufacture of evaporated and sweetened condensed milk is that it must be heat stable (i.e., must not coagulate when subjected to intense heating). There are three different classes of NFDM based on their relative concentrations of undenatured whey protein nitrogen and expressed as whey protein nitrogen index (WPNI). The American Dairy Products Institute (ADPI, 2002) classification of NFDM is given in Table 13.5.

The most preferred NFDMs used for sterilized milk products are medium- or high-heat NFDM that are manufactured from skim milk that has been preheated to 82°C for 3 minutes or 82°C for 30 minutes, respectively, before evaporation and drying. Low-heat NFDM is more suitable for nonsterilized products such as SCM. After reconstitution of the dried milk in water and recombined with milk fat or vegetable fat by high shear mixing and homogenization,

Table 13.5. Heat Classification of Nonfat Dry Milk

Class	Whey Protein Nitrogen (mg/g)
Low heat	Not less than 6.0
Medium heat	1.51–5.99
High heat	Not more than 1.5

the processing steps similar to those described above for evaporated milk and sweetened condensed milk are followed.

QUALITY ASSESSMENT

The quality of evaporated and sweetened condensed milks depends on the quality of the starting raw ingredients and processing conditions—including adherence to strict sanitation and good manufacturing practices. In the United States, raw-milk quality must follow Grade A standards specified by the Pasteurized Milk Ordinance (PMO; FDA, 2003) as follows:

Temperature	Cooled to 10°C (50°F) or less within 4 hours or less after the commencement of the first milking, and to 7°C (45°F) or less within 2 hours after the completion of milking, provided that the blend temperature after the first milking and subsequent milkings does not exceed 10°C (50°F).
Bacterial limits	Individual producer milk not to exceed 100,000 CFU/mL prior to commingling with other producer milk. Not to exceed 300,000 CFU/mL as commingled milk prior to pasteurization.
Drugs	No positive results on drug residue.
Somatic cell count*	Individual producer milk not to exceed 750,000/mL.

Quality tests are done to assess the following:

Microbiology: The microbiological standards for SCM are as follows:

Standard plate Count (SPC) 1,000 CFU/g
Coliform 10 CFU/g
Yeast 5 CFU/g
Mold 5 CFU/g

The recommended microbiological testing methods for SPC, coliform, yeast, molds, and thermophilic and thermophilic counts are described in the Standard Methods for the Examination of Dairy Products (APHA, 1992).

Other Tests: There are several methods for assessing the quality of evaporated milks and other concentrated milks. Some of the more frequently used methods are described below. For detailed description of the methods as well as other methods, refer to Methods for Quality Assessment of UHT milks (New Zealand Dairy Research Institute, 1997).

Gelation: This may be detected by visual inspection and is often rare within 6 months of production. Gelation may be indicated by wheying off and shrinkage of the gel away from the wall of the container or by failure of the surface to flow when the container is tipped or by the presence of curdy lumps when the sample is disturbed.

Fat separation: This is evidenced by cream layer on top of the product. Fat separation can be assessed by a visual subjective method or by gravimetric (weight test) or by fat emulsification methods. When the visual method is used, a cream layer may not be noticeable in well-homogenized milk before 2 months of age of the product.

Sedimentation: A subjective visual test is used to determine sedimentation of the product at the bottom area of package after pouring out the product and comparing the sediment on the bottom of the container with an internal standard chart developed with different degrees of sediment. Alternately, sedimentation is calculated as the weight difference of the container before and after rinsing off sediment.

Viscosity: The viscosity of concentrated milk is higher than that of normal milk and is in the range 15–60 cP. It is affected by fat and protein contents of milk and by processing conditions such as heat treatment. Typically, the test is done using a Brookfield viscometer equipped with spindle 2 at 60 rpm. The temperature of the concentrate is kept at 40°C ± 0.5°C.

Coffee sediment test: In the coffee sediment test, the addition of evaporated milk to hot coffee is simulated and the quantity of sediment formed after centrifugation is measured. The results of the test are influenced by the composition of coffee, pH, temperature, and quantity of water used to make the coffee. Therefore, it is important to standardize the method including the type and the brand of coffee used to get reliable results.

Coffee whitening: The coffee whitening test is similar to the coffee sediment test but instead of centrifuging out the sediment, the color is measured using a reflectance colorimeter. In addition to the precautions listed for the sediment test above, it is important that the coffee be freshly made as the color of coffee darkens on standing.

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14

Dry Milk Products

Mary Ann Augustin and Phillip Terence Clarke

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INTRODUCTION

Milk and milk products are supplied as fresh milk products, concentrates, and dry products. Milk powders may be used as a substitute for fresh milk and concentrates. The conversion of a liquid dairy stream into powder enables the supply of milk solids in a convenient stable form. Dried dairy products are produced by the dehydration of liquid milk streams or fractions of dairy streams. Manufacture of skim milk powders and full-cream milk powders enabled the development of the recombined milk and milk product industry which started in the middle of the twentieth century and is now well established with a turnover of more than U.S.\$5–6 billion in 2002

(Sanderson, 2004). A number of other powders such as whey and whey protein concentrate powders, protein powders (milk protein concentrates, caseins and caseinates, whey protein isolates), buttermilk powders, and cream powders are available in the market for both the recombined dairy industry and the wider food industry.

The milk powders can be made into a range of reconstituted and recombined dairy products including recombined pasteurized and UHT milks, in-can sterilized concentrated milk, sweetened condensed milk, cream, ice cream, fresh cheese, yogurts, and dairy desserts. Milk powders are also used as ingredients in many manufactured food products. In food applications, the components of the milk products (e.g., fat, protein, lactose, milk salts) contribute to the desired properties of the food product.

Milk powders can play many functional roles when incorporated into food products. These have traditionally included milk powders in a nutritional role as milk is a good source of nutrients and a physical functional role where the milk powder imparted texture and contributed to the sensory appeal of the final food product. More recently, with the development of the functional food industry and the recognition that milk contains a number of bioactive components, users of milk ingredients are also interested in the physiological role of milk ingredients in manufactured food products. Milk powders can also serve as delivery vehicles for bioactive ingredients.

Developments in milk powder technology and a better understanding of the physical and chemical changes to milk as water is removed has led to improved consistency of milk powders and allowed differentiation of milk powder properties. Different aspects of milk powder manufacture and their

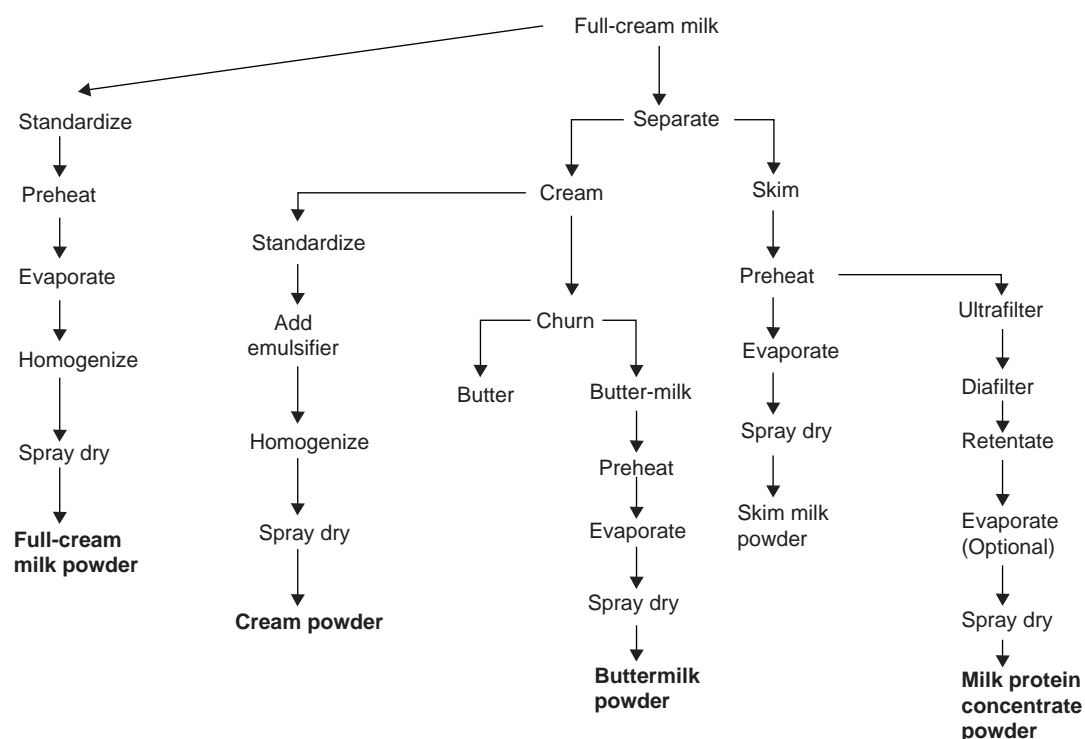


Figure 14.1. Flow chart for manufacture of selected dry milk products.

applications have been discussed by various authors (Augustin et al., 2003; Augustin and Margetts, 2003; Kelly, 2006; Kelly et al., 2003; Singh and Newstead, 1992; Tong, 2001). In this chapter, the technology of milk powder manufacture, the properties of milk powders, and their applications are discussed.

MILK POWDER PROCESSING

The unit processes in the manufacture of dry milk products include standardization of the milk streams, preheating, concentration, homogenization, and drying. The generalized processes for the manufacture of a selected range of dry milk products, starting with full-cream milk as the raw material, are shown in Figure 14.1. The approximate compositions of the major traditional milk powder products are as follows: skim milk powder (36% protein, <1% fat, 51% lactose, 8% ash water, 3–4% moisture); full-cream milk powder (26% protein, 27% fat, 38% lactose, 6% ash, moisture 3%).

The usual process for the manufacture of full-cream milk powder involves a preheat treatment of the full-cream milk, followed by thermal evaporation, homogenization, and spray drying. An alternative process for the manufacture of full-cream milk powder involves the separation of full-cream milk into skim milk and cream followed by heat treatment of each of these streams separately. The skim milk is given a low heat treatment and concentrated by evaporation while the cream is subjected to a high heat treatment. The skim concentrate and cream are then combined back into a full-cream milk concentrate and spray dried. Full-cream milk powders produced by the alternative processes have similar flavor and physical characteristics to powders prepared by the traditional process. Fouling of the evaporators is expected to be reduced with the use of the alternative process (Hols and Van Mil, 1991).

It is essential that milk powders be made from high quality milk and that their compositional and technical specification relate to end-use requirements (Jensen, 1990).

STANDARDIZATION OF DAIRY STREAMS

The standardization of dairy streams is essential to insure that the dry milk products meet the required compositional specification for dry milk products. Traditionally, skim milk powder is defined as the product resulting from the removal of fat and water in milk to result in a product which contains not more than 5% moisture and 1.5% fat. This definition requires the lactose, milk proteins, and milk minerals to be in the same relative proportion as in the fresh milk (American Dairy Products Institute, 2002).

With the advent of legislation that allows for standardization of skim milk powder, some specification now stipulate a level for the minimum protein content in skim milk powder. The milk products that can be used for standardizing the protein content of milk include (a) milk retentate—obtained by concentration of milk, partly skimmed milk or skim milk, by ultrafiltration (b) milk permeate—obtained by using ultrafiltration to remove milk proteins and milk fat from milk, partly skimmed milk or skim milk, and (c) lactose (Codex Alimentarius, 1999).

The incorporation of protein content into the specification for milk powders is a logical extension of traditional milk powder specification because protein has a major influence on the attributes of the milk powder in many applications. Full-cream is standardized to a desired solids nonfat (SNF): fat ratio to obtain powder that meets a minimum of 26% milk fat. Buttermilk powder is obtained by drying buttermilk which is a by product of butter manufacture. It should have a minimum protein content of 30% and a fat content greater than 4.5% (ADPI, 2002). One of the distinguishing features of buttermilk powder compared to skim and full-cream milk powders is its high amount of phospholipids. Buttermilk powder also has a stronger dairy flavor.

Specification for dry milk products including some additional quality factors are shown in Table 14.1. Although milk powder is a microbiologically stable product, the microbial quality of the raw milk influence the shelf-stability of the powder and it is essential to use good quality milk to manufacture powders.

PREHEATING

Milk streams are given a heat treatment prior to concentration. The heat treatment is essential to obtain dry milk products with good microbiological quality. The heat treatments applied to skim milk vary,

ranging from a low heat (typically 72°C for 15 seconds) to medium heat (75°C for 1–3 minutes; 85–105°C for 1–2 minutes) to high heat (85°C for 30 minutes; 90°C for 10 minutes; 120–135°C for 1–2 minutes).

The preheat treatments are also used to achieve a desired level of denaturation of the whey proteins. The heat treatment applied prior to concentration is the prime determinant of the extent of whey protein denaturation during skim milk powder manufacture, as there is minimal heat damage occurring during the concentration and drying stage (Singh and Newstead, 1992). The extent of whey protein denaturation has a significant influence on the physical functionality of the milk powder in its end-use. This has led to the development of a range of indices for classifying skim milk powders based on heat treatment.

The whey protein nitrogen index (WPNI) has traditionally been used as the indicator of the heat treatment used in skim milk powder manufacture (Table 14.2). However, there are seasonal and other natural variations in the protein composition (i.e., whey protein and casein content) of the raw milk supply which can affect the WPNI data. Specifically, a fixed heat treatment can result in different extents of whey protein denaturation in milk when there are differences in the amount of whey protein in the raw milk or a change in the ratio of casein:whey protein (Jensen, 1990).

Other indicators of the extent of whey protein denaturation, such as the casein number, heat number, thiol number, cysteine number, and furosine content (Tong, 2001; Wilcek, 1990), sulphhydryl content and absorbance at 340 nm (Guingamp et al., 1999) have been also suggested as alternative measures of the extent of heat treatment for skim milk powders (Table 14.2). However, the WPNI method remains the guide that many manufacturers still use for classifying skim milk powders.

Full-cream, buttermilk, and cream powders are not generally given a heat classification. Nevertheless, the trends in WPNI do relate to the intensity of the heat treatment. High heat treatments have been shown to extend the shelf life of full-cream milk powder (Baldwin and Ackland, 1991). The heat treatment inactivates lipase present in milk and also develops the natural antioxidant activity of the milk components. Heat treatment causes exposure of the sulphhydryl groups and enhances the Maillard reaction. Both these events contribute to an increased oxidative stability of the milk powder (McGookin and Augustin, 1997; Taylor and Richardson, 1980).

Table 14.1. Specifications for Dry Milk Products

Product	ADPI ^a	Codex Alimentarius
Skim milk powder		
Fat	Max. 1.25%	Max. 1.5%
Water	Max. 4.0%	Max. 5.0%
Protein		Min. 34% ^b
Titrateable acidity	Max. 0.15%	Max. 18 ^c
Solubility index	Max. 1.2 mL	Max. 1.0 mL
Bacterial estimate ^b	Max. 10,000 per g	
Scorched particles	Max. Disc B (15.0 mg)	Max. Disc B
Full-cream milk powder		
Fat	Min. 26%, Max. 40%	Min. 26%, Max. 42%
Water	Max. 4.5% ^d	Max. 5.0%
Protein		Min. 34% ^e
Titrateable acidity	Max. 0.15%	Max. 18 ^c
Solubility index	Max. 1.0 mL	Max. 1.0 mL
Bacterial estimate	Max. 10,000 per g	
Scorched particles	Max. Disc B (15.0 mg)	Max. Disc B
Buttermilk powder		
Fat	Min. 4.5%	
Water	Max. 4.0%	
Protein	Min. 30%	
Titrateable acidity	0.10–0.18%	
Solubility index	Max. 1.25 mL	
Bacterial estimate ^c	Max. 20,000 per g	
Scorched particles	Max. Disc B (15.0 mg)	
Cream powder		
Fat		Min. 42%
Water		Max. 5.0%
Protein		Min. 34% ^b

^a American Dairy Products Institute (2002) specification for Extra Grade.

^b With coliforms not greater than 10 per g.

^c As mL 0.1N NaOH/10 g solids non-fat.

^d As determined by weight of moisture on a milk solids non-fat basis.

^e As a proportion of milk solids non-fat.

Table 14.2. Heat Classification of Skim Milk Powder

Milk Powder Class	Whey Protein Nitrogen Index ^a	Cysteine Number ^b	Thiol Number ^b	Heat Number ^b
Extra low heat		24–31		
Low heat	≥ 6	31–38	<7.5	≤ 80
Medium heat	1.51–5.99	38–62	7.5–13.3	80.1–88
High heat	≤ 1.5	>62	>13.3	>88.1

^a From American Dairy Products Institute (2002).

^b IDF specifications Wilcek (1990).

CONCENTRATION

In the production of skim and full-cream milk powders, the milk stream is thermally concentrated, typically to 45–50% total solids, prior to drying in a spray dryer. A multistage falling film evaporator is normally used. The evaporation is performed under vacuum. There is little additional heat damage during concentration after the preheat treatment of the milk (Oldfield et al., 2005). This is because the residence times in each stage of the evaporator are short (~60 seconds). The maximum temperature the milk is exposed to is 72°C in the first stage, with lower temperatures in subsequent stages (Singh and Newstead, 1992). As the thermal evaporation of water is a cheaper process than removal of water by spray drying, it is desirable to have concentrates with high total solids content fed into the dryer.

The viscosity of concentrates fed into the dryer has an influence on the properties of milk powder, in particular, increasing viscosity leads to a significant reduction in the solubility of the powder (Baldwin et al., 1980). High viscosity affects the efficiency of the drying process (Bloore and Boag, 1982). Hence, viscosity needs to be controlled and monitored. The viscosity of the concentrate increases with a higher degree of concentration and a longer holding time of the concentrate. The viscosity of the concentrate is affected by the natural variations in milk composition and by the heat treatment applied prior to concentration.

A high protein concentration in milk can significantly increase the viscosity of the concentrate (Bloore and Boag, 1981). Increasing heat treatment, resulting in a higher extent of whey protein denaturation, also increases the viscosity of the concentrate. Recent work on reconstituted whole milk concentrates confirms that solids content, heating, and storage temperature affected their rheological behavior—with Newtonian behavior observed at lower total solids, non-Newtonian behavior occurring at higher total solids, and higher heating temperatures promoting non-Newtonian behavior at lower solids (Binh et al., 2007).

It has been suggested that the total solids of the concentrate can be increased to >50% when milks are exposed to low or medium heat treatments prior to concentration but should not exceed 50% when high heat treatment of milk is applied (de Vilder and Moermans, 1983). However, others have demonstrated that increasing the solids content can pose potential problems. Jensen and Hansen (1974) found

that although increasing the solids of full-cream milk concentrates from 43 to 49% did not affect the initial solubility of the powder produced, the loss in solubility was more pronounced during 12 months storage at 30°C when concentrates had a higher content of solids.

The initial pH of single-strength milk is pH 6.7 (i.e., the natural pH of milk) and it decreases on concentration. Concentrated milk at 20% total solids has a pH ~6.45. Further concentration to 45% total solids reduces pH even further and at this concentration the pH is ~0.5–0.6 units lower than the pH of single-strength milk (Bienvenue et al., 2003). This is primarily because of the change in the mineral salt equilibria of milk as the milk is concentrated by water removal. Concentration causes transfer of calcium and phosphate to the colloidal phase of milk (le Graet and Brule, 1982) causing a re-establishment of the mineral salt equilibria, with the release of hydrogen ions. The high solids and the lower pH of evaporated milk concentrates make them more susceptible to aggregation.

Milks may also be concentrated by membrane processing. This method of concentration is used for the production of milk protein concentrates (Fig. 14.1). In contrast to the removal of water only by thermal evaporation, concentration using membranes results in the fractionation of the milk's components. The partitioning of the milk components depends on many factors including the size of the membranes, the extent of ultrafiltration and diafiltration and the conditions (e.g., pH of feed, temperature) used for these separation processes. Membrane processing leads to concentration of the milk protein in the retentate streams which are subsequently dried to produce milk powders with higher protein content than traditional skim milk powder.

Milk protein concentrate (MPC) powders with varying protein contents (40–85% protein) have been made by ultrafiltration and/or diafiltration or evaporation of the ultrafiltrate retentate prior to drying. Milk protein concentrate powders may be made as low or high heat products (Getler et al., 1997; Huffman and Harper, 1999). The total solids of the concentrate that can be fed into the dryer depends on the protein content of the MPC produced but is much lower than that used in the production of skim milk powders. The high protein and low lactose content of the concentrates prepared by ultrafiltration/diafiltration leads to the higher viscosity. This limits the solids content of the concentrates that can be fed into the dryer.

HOMOGENIZATION

Homogenization of the full-cream milk concentrate prior to spray drying is a routine step in the traditional process for manufacture of full-cream milk powder (Fig. 14.1). During homogenization, the milk concentrate is fed under high pressure through a small orifice which disrupts the native milk fat globule. The size of the globule is reduced and this is accompanied by an increase in the surface area of the fat. The natural milk fat globule membrane is insufficient to cover the increased area and a new membrane is formed, comprising a mixture of the original milk fat globule membrane and adsorbed milk proteins.

The purpose of homogenization is to decrease the surface free fat in the milk powder (de Vilder et al., 1979). Generally, full-cream milk powders are manufactured to obtain a low level of free fat in powder. This is because a high surface free fat ($>2\%$ of powder) in full-cream milk powders is undesirable as fl wability, wettability, and storage stability of the powder are adversely affected.

DRYING

In this stage, dryers are used to remove water from a milk concentrate to produce a shelf-stable product. Early commercial milk powder drying plants used roller dryers. The concentrate was fed over rotating steam or oil-heated drums to evaporate the water from the concentrate. The resultant sheet of powder was then powdered in a hammer mill to a predetermined size. The powders produced have sharp edges and are made up of compact particles (Caric and Kalab, 1987). Except for the manufacture of full-cream milk powder with high free fat content for the chocolate industry where some roller dryers are used, most industrial milk powder plants today use spray dryers (Fig. 14.2).

For spray drying, milk concentrates are fed into dryers with positive displacement pumps. The concentrate is atomized, using a rotary atomizer or a nozzle, and small droplets of concentrate are obtained. Water is rapidly evaporated from the droplet surface when it is initially mixed with the hot air in the drying chamber. The resultant dried powder particles are separated from the drying air by cyclones, collected and packaged. Spray-dried milk powders have a globular shape, with a convoluted surface and a porous structure. The method of atomization used affects the microstructure of the powder, with nozzle atomization resulting in lower occluded air and higher pow-

der bulk density (Caric and Kalab, 1987; Tong, 2001). Although the inlet air temperature of the dryer is high ($>170^{\circ}\text{C}$), there is minimal heat damage to proteins during spray drying, because of evaporative cooling, as water is removed rapidly and the particle is dried. The temperature of the particle is low ($<70^{\circ}\text{C}$) until the milk powder droplet is dried. The outlet air temperature of the dryer has the greatest effect on heat damage as this is the temperature the dried powder particle approaches (Singh and Newstead, 1992). Lactosylation of milk proteins, which is the conjugation of lactose to protein and related to heat damage, can occur during skim milk powder manufacture. It is promoted with the use of high outlet air temperatures during drying (Guyomarc'h et al., 2000).

The operating conditions of spray dryers can affect the solubility characteristics of milk powders. The detrimental effects of high temperature drying conditions are more pronounced during manufacture of high protein milk powders compared to standard skim and full-cream milk powders. In the manufacture of milk protein concentrate powders (75% protein in powder) impaired hydration properties were obtained when the outlet temperature of the spray dryer was increased from 65 to 95°C (at a constant inlet temperature of 250°C). Detrimental effects on hydration properties of MPC powders were also obtained with an increase in the inlet temperature (de Castro and Harper, 2001; de Castro-Morel and Harper, 2003).

There are a number of comprehensive descriptions of the engineering aspects of spray drying available (Masters, 1991, 2002; Mujumdar, 1995; Pisecky, 1997). Further developments in process control techniques for optimization of milk powder production require an improved understanding of the thermodynamic properties of the milk and concentrate and the interactive effects of time, temperature, and shear during the course of milk powder manufacture (O'Callaghan and Cunningham, 2005).

INSTANTIZATION

Instantization is carried out to improve wettability, dispersibility, and the free-fl w properties of milk powders. Instantizing may be achieved by returning the fine powder particles into the drier, close under the rotary atomizer or spray nozzles, so that the particles aggregate to form agglomerated powder (Fig. 14.3).

Lecithin is often used to improve the properties of instantized milk powders (Sanderson, 1978). A traditional method of application involves dissolving the



Figure 14.2. Spray dryer. (Reproduced with permission of Niro Inc.)

lecithin in butter oil and spraying it over the agglomerated milk powder, either internally or in a fluidized bed external to the dryer. The process requires strict adherence to controlled temperatures during powder manufacture to allow both the hydrophilic and lipophilic components of the lecithin to interact with the free fat in a molten state. Hence, when using a wetting agent such as 30–50% lecithin in an oil solvent, the mixture should be 60–65°C, the powder temperature must be a minimum of 50°C and the powder must be fluidized for at least 5 minutes at 45°C (Pisecky, 1997).

Recently, an alternative *in situ* process for lecithination of skim milk powders has been reported. In

this process, lecithin is added to the feed introduced into the spray dryer. This approach was based on the finding that the surface of a spray-dried milk powder is dominated by surface-active species and that the most rapidly adsorbing surface-active agent dominates the composition of the surface of a powder particle (Millqvist-Fureby and Smith, 2007).

PROPERTIES OF MILK POWDERS

Powders in the market must meet general standard specification for trade (Table 14.1). This is the minimum requirement. The composition and microbiological quality of the milk powder, though essential

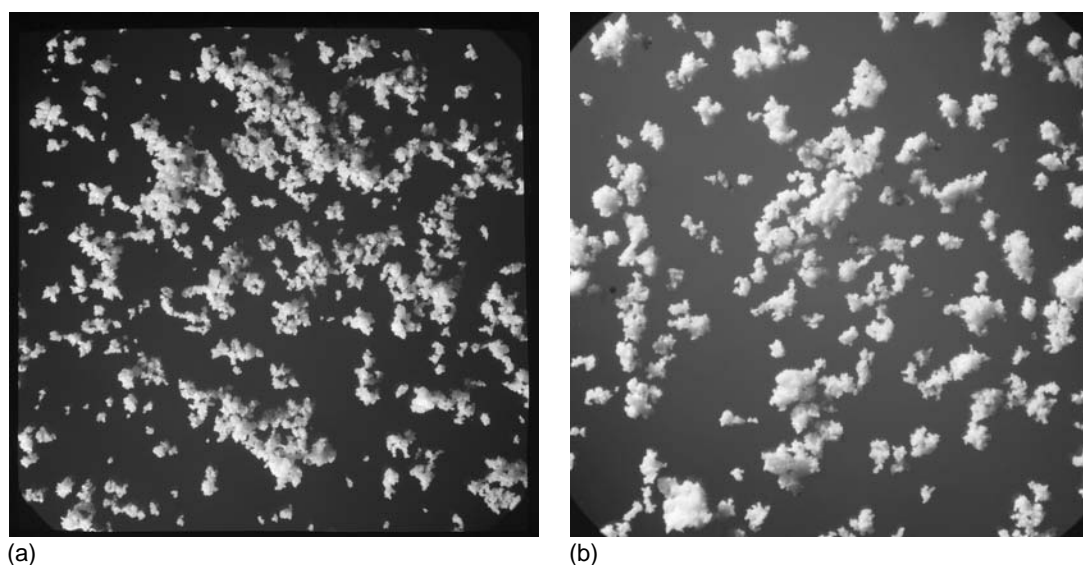


Figure 14.3. Agglomerated milk powders. (a) Skim milk powder; (b) Full-cream milk powder. (From Pisecky, 1997. Reproduced with permission of Niro Inc.)

attributes, do not always reflect their performance in their intended application. A number of tests have been developed for further characterization of milk powders. These may be used as quality control measures or as a guide for assessing the physical functionality of the powders.

PHYSICAL CHARACTERISTICS OF POWDERS

The physical characteristics of milk powders need to be understood if they are to be fit for the intended application.

Bulk Density

Bulk density is a measure of the weight of powder that can be contained in a set or known volume. It is also referred to as packing density and can be expressed in g/cm^3 , kg/m^3 , or g/100 mL (Pisecky, 1997). The bulk density is a consideration for packaging particularly when transport is being costed on a volume basis. Bulk density also has an influence on other aspects of powder functionality including dispersibility, wettability, and instantizing.

The bulk density of milk powders is measured on a known weight of powder transferred to a measuring cylinder. The initial volume is the poured bulk

density. The cylinder is then tapped, usually 100 times and then a further 525 times to give the loose and final bulk density, respectively. There are also variations on this number of taps. There are many manual (American Dairy Products Institute, 2002) and automated (e.g., Stampvolumeter) methods for this determination and the method must always be quoted when giving determinations. Typical results for skim milk powder are in the range of 0.58–0.68 g/mL . For full-cream milk powder, the bulk density is 0.56–0.66 g/mL for nonagglomerated powder and 0.45–0.52 g/mL for instantized powder.

During powder manufacture, many variables can play a part in the final bulk density of the powder produced including the concentrate characteristics, atomization methodology, drying parameters, and the extent of whey protein denaturation. The parameters that determine the final bulk density are the occluded air (i.e., the amount of air entrapped within the individual powder particles), the interstitial air (i.e., the amount of air or space between the powder particles themselves), and the distribution of the size and shape of the powder particles. Powders with a range of particle sizes give a higher bulk density than a powder with a narrow particle size distribution. With respect to the effects of shape, powder particles that are more uniform and smoother give rise to higher bulk densities.

Flowability

Powder flowability is an important attribute of milk powders in the area of transport, packaging, and handling.

The measurement of flowability is particularly difficult. Measurements can be carried out using one of the sophisticated analytical instruments on the market (e.g., Hosakawa micron powders tester, Aeroflow powder flowability analyzer) or simple tests involving measurement of powder flow through a funnel, down an incline or the angle of repose after forming a powder pile under controlled conditions. An alternative method involves using a rotating drum developed by Niro and this gives results for a wide range of powders (Pisecky, 1997). Measurements using this drum method indicate that the flowability of agglomerated skim milk powder > agglomerated full-cream milk powder > instant full-cream milk powder > ordinary full-cream milk powder.

Flowability is a very complex issue which is influenced by many factors. Flowability is improved by the use of flow additives, minimizing the amount of fines increasing the particle size, and by having more spherical and smooth particles. An increase in moisture, particularly surface moisture and or fat content, particularly free fat, has a detrimental effect on flowability.

Reconstitutability

The ability of a powder to be reconstituted is dependent on its ability to be wet, to sink, to disperse, and finally to dissolve. Complete dissolution is important for functionality of powders in an application.

Wettability. In order for a powder to be reconstituted it must first be penetrated by the water in which it is being dissolved. The powder must be able to overcome the surface tension between itself and the water in the first instance.

A typical method for the measurement of wettability consists of systematically placing a weighed amount of powder on the surface of a known volume of water at a set temperature and then measuring the time taken for all of the powder to disappear below the surface of the water (Pisecky, 1997).

The degree of wettability is strongly influenced by several factors; two of the most significant are the free fat content of the powder and the state of the lactose. Under some conditions of manufacture or storage, the amorphous lactose may be changed to a crystalline

state and damage the fat globule membrane (Kelly et al., 2003), causing an increased level of free fat in full-cream milk powders. One way to overcome the problem associated with reduced wettability is to add surfactants such as lecithin to the powder. Wettability is also reduced when there is an increase in interstitial air between the powder particles.

Sinkability. A closely aligned attribute to wettability is the sinkability of powders. Once the powder particle has been initially wetted it then must be able to sink into the water for complete dispersion and solubility.

Sinkability may be measured by recording the time required for the disappearance of powder from the water surface after a portion of a milk powder has added to water and stirred with an impeller under fixed conditions (Schober and Fitzpatrick, 2005).

The conditions used for reconstitution influence the sinkability of a powder. The creation of a vortex and maintaining it during reconstitution is crucial for sinkability. The particle density is also an influencing factor in sinkability in that the heavier the particle per unit volume the more likely it is to sink. Thus, low interstitial air content is a prerequisite to good sinkability.

Dispersibility. During the process of dissolving powders the agglomerates need to instantly disintegrate into single particles to facilitate wettability and dissolution.

The dispersibility of powders is measured by systematically placing a weighed amount of powder (typically 10 g) onto the surface of a set amount of water (250 mL at 25°C), stirring the solution for a set time in a rotational pattern, sieving the contents and after drying, weighing the residue. The dispersibility is reported in terms of the mass of the test portion and the values for water content and total solids (Pisecky, 1997).

To facilitate dispersibility, the agglomeration process must be controlled to produce few if any agglomerates >250 µm in size.

White Flecks. White flecks are particles that remain undissolved in a milk solution after reconstitution. They can be observed when the solution is spread to form a thin film for example, on the back of a spoon after the solution has been allowed to stand for several minutes. The white fleck can also form a surface layer. They tend to be more prevalent when high total solids solutions are prepared. Although

white fleck tend to be rather soft they can cause physical problems when the reconstituted milks are used in processing operations, as they can clog filter and sieves and can be visibly undesirable in the final product.

PHYSICAL FUNCTIONALITY OF POWDERS

When a milk powder is used in an application either as the primary or secondary ingredient it imparts physical attributes to the final products which are often essential for the success of the application. These physical attributes include solubility, heat stability, gel forming, thickening or viscosity control, foaming, and binding characteristics.

Solubility

Solubility is a prerequisite for most other functional attributes because if the powder cannot be efficiently solubilized then it cannot impart the desired attribute effectively. If the powder is not completely dissolved it can cause problems in processing such as clogging of filter and loss of material due to sedimentation, and there is also the need for subsequent removal of undissolved material.

Powders are tested for insolubility by determining the amount of insoluble material remaining after a prescribed method for dispersion of the powder at a nominated total solids concentration at defined temperature and mixing techniques. The most common of these is the method used by the American Dry Products Institute (2002). The insoluble material is usually made up of denatured protein (typically β -lactoglobulin) complexed with casein and lactose in various ratios.

There is a range of factors that are known to contribute to the formation of insoluble material in milk powder. The most critical factor controlling the insolubility of powders is the temperature of the particle during the removal of water in the dryer when the moisture content is between 10 and 30%. Other factors that contribute to insolubility include the pre-heat treatment of the milk during manufacture where higher temperatures more often lead to higher insolubility, type of dryer used (with roller dryers being worse than spray dryers), the configuration of the spray dryer (such as the type of atomization), and single-stage versus multi-stage drying, and the physical properties of the concentrate prior to drying (e.g., viscosity).

Another very critical factor that influences powder solubility is the temperature at which the milk powder is reconstituted. Solubility is usually highest between 40 and 60°C, particularly when preparing a high solids reconstituted concentrate from powder.

Heat Stability

During the processing of most products heat is used in some form. Therefore, milks reconstituted from powders, when incorporated into a product, will be subjected to heat of various degrees. During heating the milk is required to not unduly thicken or coagulate depending on the application. The susceptibility to heat is magnified in concentrated milk solutions such as evaporated milk.

Several alternative methods have been developed in an attempt to measure heat stability of powders in general and also for specific end-uses. Typical of the common methods is measuring the coagulation time of a milk solution at a specific total solids, at temperatures in the range of 120–140°C. Another method using time coagulation criteria is the ethanol stability test (Horne and Parker, 1980) where mixtures of reconstituted milk with various amounts of ethanol are used. The drawback of these tests is that they measure the heat stability of the milk solution under defined conditions that do not directly predict the heat stability in the intended application where the environment can be quite different.

The closer the conditions of the test are to the conditions used in the intended application, the better the correlation. An objective laboratory-scale method for examining the suitability of skim milk powders for recombined evaporated milk manufacture has been developed. The method involves heating concentrated milk to 120°C/13 minutes, and measuring the viscosity of the sterilized concentrated milk. This method has proven to be a good guide to the stability of recombined evaporated milk during retorting (Kieseker and Aitken, 1988). This laboratory-based method is an alternative to costly and time-consuming pilot plant trials.

Many factors affect the heat stability of milk powders. Heat treatment of milk applied during powder manufacture has been used to manipulate the heat stability of milk powders. A high heat treatment of milk which results in a high level of whey protein denaturation (i.e., a low whey protein nitrogen index, WPNI < 1.5 mg denatured whey protein/g powder) is desired for adequate heat stability of concentrated milks. However, reliance solely on WPNI to assess

the ability of concentrated milks to withstand subsequent heat treatment is not recommended. This is because other factors (e.g., pH, mineral balance of milk) can have a more significant effect on heat stability.

Viscosity

Milk powder is used to influence the viscosity of products in a range of applications. Viscosity control is particularly important in high solids products such as recombined sweetened condensed milk.

The viscosity of milks reconstituted from milk powders is usually measured by a method aligned with the application in which the powder is intended for use. A single strength solution at a specific temperature is a good starting point for many applications. However, in applications where a higher than single strength solution is to be used then specific tests must be undertaken which mimic the environment of the application. For example, tests for assessing the suitability of milk powders for recombined sweetened condensed milk involves making a mixture of skim milk powder, sugar, and water in the same proportions as the final product, heating the mixture under standardized conditions that are representative of the process used in industry and measuring the viscosity of the final mixture (Kieseker and Southby, 1965; Weerstra et al., 1988). Alternatively pilot-scale trials can also be carried out to test suitability of powders for recombined sweetened condensed milk applications.

The major factor that influences the viscosity of recombined sweetened condensed milk is the preheat treatment of the skim milk applied during powder manufacture. Generally, medium heat milk powders are suitable for this application. Increasing the extent of whey protein denaturation in the powder to >50% results in marked increases in viscosity of recombined sweetened condensed milk (Cheng et al., 2000).

Gelling

Milks do not gel at their natural pH. However, they gel on acidification as the pH is reduced to pH 4.6. Gelation is a consequence of the reduction of the charges on the milk proteins as the pH approaches the isoelectric point.

Acid milk gels may be formed by addition of an acidulant such as glucono- δ -lactone or with the use of cultures as in the production of yoghurt. The

strength of milk gels may be measured using standard texture analyzers (e.g., Instron, TA-XT2 Texture analyzer).

The acid gelation properties of milk are affected by the milk composition and the heat treatment applied during powder manufacture. Firmer gels are made with milks that have been given a high heat treatment. Improved yoghurt properties are obtained with increasing whey protein denaturation in milk powder (Augustin et al., 1999).

Foaming and Emulsifying Properties

Milk powders offer good emulsifying and foaming capabilities that are required for some applications. In skim milk powder, the main surface-active components are the milk proteins, whereas in full-cream milk powders, there is also the phospholipid component of the milk fat globule membrane. Caseins, whey proteins, and phospholipids are able to stabilize the air/water interface of air bubbles in foams and the oil/water interfaces of fat droplets in emulsions due to their amphiphilic properties.

Measurement of foaming capacity can be undertaken by simple methods using domestic mixers with milk solutions at set times and temperatures and measuring the resultant foam generated (Phillips et al., 1987). The emulsion capacity of milk powder solution can be determined by the principle of pumping oil into a protein solution while homogenizing and monitoring the electrical resistance of the solution. A decrease in electrical resistance is observed when the solution changes from an oil-in-water emulsion where water is the continuous phase to a water-in-oil emulsion with oil as the continuous phase. A typical example of this method is given by Vuilleumard et al. (1990).

The foaming and emulsifying properties of milk powders can be influenced by their composition, processing treatments applied to the milk as well as the conditions used for the formation of emulsions and foams. Physical changes can be made to the powder morphology to enhance the foaming capacity. This may be done by manufacture of high occluded air in powders by altering processing variables or by injecting air into the concentrate prior to drying.

STORAGE STABILITY OF POWDERS

The physical properties of milk powders may be altered when they are stored. The storage of powders at high temperature and humidity accelerates the

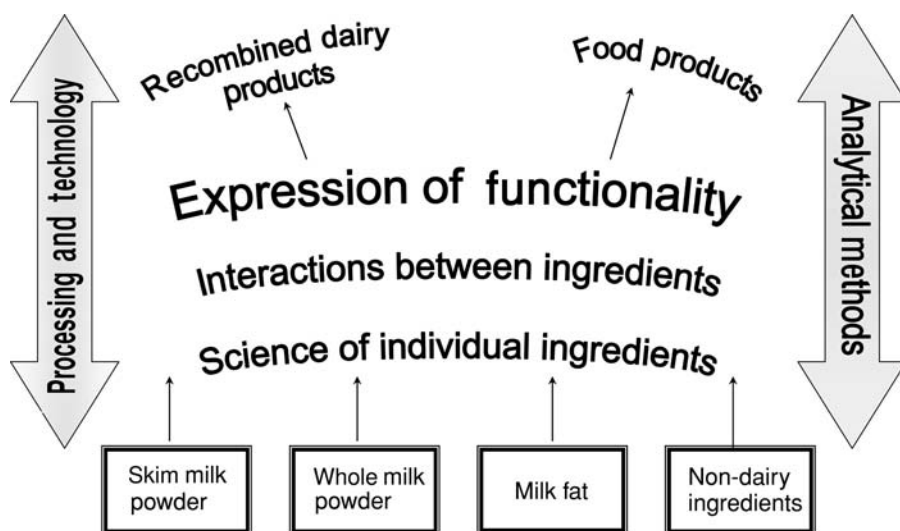


Figure 14.4. Roadmap for using milk powders.

damage to milk powders. There are a number of phenomena and reactions that cause the quality of milk powders to deteriorate. These include lactose crystallization, chemical, and enzymic reactions. The consequences of these reactions are a loss of solubility and impairment in many of the other physical (e.g., increased tendency to cake and reduced flowability) and functional attributes (e.g., gelling, emulsifying, and foaming) of milk powders. The effects of ageing on the properties of milk powders have been recently reviewed (Thomas et al., 2004).

APPLICATIONS OF MILK POWDERS

Milk powders are used in a range of applications. The physical functional attributes of powders govern their ability to contribute to attributes of the final product. Figure 14.4 depicts the roadmap for using milk powders. Success in using milk powders requires an understanding of the properties of the individual powder ingredients and how their functionality is expressed in the final food product. This is because the milk powder components can interact with other ingredients when formulated and processed into a final recombined dairy product or food product. For the purpose of quality control, it is beneficial for milk powder suppliers to work with end-users of their ingredients and to understand how their products will

be formulated and processed. This allows the development of more appropriate fitness-for-purpose specification and methods for testing the attributes of milk powder ingredients.

Milk powders can also impact on flavor and color of products. Buttermilk is sometimes used as a partial replacer of other milk powders to improve the flavor of dairy products. In some applications, a bland milk powder is desirable so as not to impart flavor tones which may be undesirable for the specific product.

RECOMBINED AND RECONSTITUTED DAIRY PRODUCTS

Early reconstitution and recombination was for the manufacture of simple products such as liquid milk, sweetened condensed milk, and evaporated milk. Today, all traditional milk products can be made from milk powder and milk-based ingredients. Figure 14.5 shows the operations involved in the process required for the production of recombined milk.

When a milk powder is simply mixed with water this is called reconstitution. For example, reconstituted skim milk and reconstituted full-cream milk are made by dispersion of a skim milk powder and full-cream milk powder in water respectively. Depending on the shelf-life required, the reconstituted milk may be pasteurized or given a high heat treatment for sterilization. When several milk ingredients are mixed

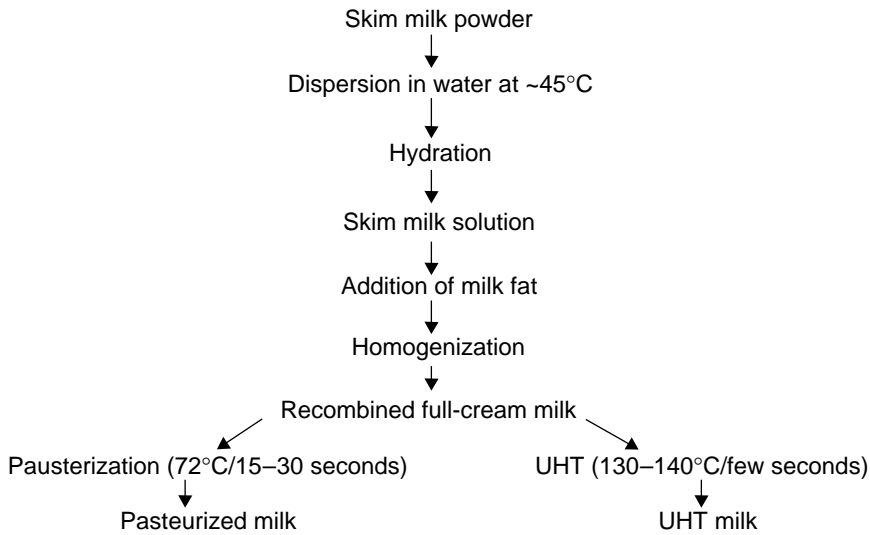


Figure 14.5. The process of recombination of milk.

for the production of a dairy product, this process is termed recombining. In this case, usually the skim milk powder ingredient is reconstituted in water and combined with anhydrous milk fat, homogenized and heat-treated. Typical recombined milk products include recombined UHT single strength milk (e.g., 12% total solids, 9% skim milk solids, 3.5% fat), recombined retort-sterilized evaporated milk (e.g., 26% total solids, 18% skim milk solids, 8% fat), and recombined sweetened condensed milk (e.g., 74% total solids, 20% skim milk solids, 8% fat, 46% sucrose). Other recombined dairy products include recombined cheese, cultured milk products (e.g., labneh, yoghurt), cream and ice cream (Jensen, 1990). When the milk fat component in a recombined dairy product is substituted with a nondairy fat (e.g., canola oil, palm oil), the resultant product is called a filled dairy product.

Milk powders are now able to be tailored to suit a vast range of applications for the manufacture of dairy products. This is because milk powder processes have the means to manipulate composition, physical, and functional characteristics. However, while the heat treatment given during skim milk powder manufacture remains the dominant factor for selection of powder for a particular end-use in a manufactured recombined dairy product (Table 14.3); other characteristics (e.g., quality of the powder) also need to be considered.

APPLICATIONS IN NONDAIRY PRODUCTS

A growing number of nondairy products are manufactured using milk powders as an ingredient. Milk powders are found in such products as meat, bakery, confectionery, chocolate, sauces, and desserts. Their functional characteristics, essential in many applications, include browning and flavor development, water binding, emulsification viscosity modification and texture. The inherent properties of milk powders may be used as a guide to powder selection for specific end-uses (Table 14.3). However, the functionality of a powder in the final application is dependent on the ingredients in the food formulation and the processing variables used in the manufacture of the final food product.

SPECIALIZED MILK POWDERS

In addition to the conventional milk powders, there is a growing range of powders that are specially tailored to provide physical, nutritional, or physiological functional roles in a food product.

MILK POWDERS FOR CHOCOLATE MANUFACTURE

When full-cream milk powder is intended for chocolate manufacture, a high level of free fat is desirable

Table 14.3. Functional Requirements of Skim Milk Powders in Selected Applications

Product	Heat Treatment of Milk Powder	Desirable Powder Attributes
Single strength milk Pasteurized milk	Low–medium heat	Reconstitutability Good flavor Emulsifying
UHT milk	Low–medium–high heat	Reconstitutability Good flavor Heat stability Emulsifying Low level of heat-stable enzymes
Concentrated milk products Evaporated milk	High heat	Reconstitutability Heat stability Viscosity
Sweetened condensed milk	Low–medium–eat	Reconstitutability Viscosity
Other recombined dairy products		
Yogurt	Low heat	Water binding Viscosity Gelling
Cream	Low–medium heat	Good flavor Emulsifying
Cheese	Low heat	Rennetability
Other products		
Ice cream	Low–medium–high heat	Foaming/whipping Emulsifying
Confectionery	High heat	Water binding Foaming/whipping Emulsifying Heat stability
Bakery	High heat	Water binding Foaming/whipping Emulsifying Gelling
Meat products	High heat	Water binding Foaming/whipping Emulsifying Gelling

as this reduces the amount of cocoa butter and surfactants needed in the chocolate formulation, as the viscosity of the chocolate mass is reduced and less energy is used for chocolate manufacture. When the traditional process is used for manufacture of full-cream milk powder (Fig. 14.1) with a typical fat

content of 26% fat, a powder with low level of free fat is obtained. However, roller-dried powders have high levels of free fat, making them more suitable for chocolate manufacture (Augustin, 2001; Reimerdes and Mehrens, 1993). Compositional factors can impact on the free fat content of milk powder. Higher

solid-fat content of the fat (Twomey et al., 2000) or of the fat content of the concentrate (Kelly et al., 2002) can lead to an increase in free fat of spray-dried milk powders.

Significant increases in free fat in powders may also be achieved by modifying the method used for manufacture. High free fat powders were obtained by (a) increasing the temperature of the concentrate fed into the spray dryer or decreasing the inlet air temperature and increasing the outlet air temperature of the spray dryer (de Vilder et al., 1976, 1979); (b) combining skim concentrate with cool cream or cream homogenized at high temperature prior to drying (Clarke and Augustin, 2005); or (c) exposing full-cream milk powder to high shear and high temperature in a twin screw co-rotating processor (Koc et al., 2003).

Comparisons between the performance of roller-dried full-cream milk powder, spray-dried with added butter oil and spray-dried full-cream milk powder with a high free fat content indicated that free fat was a major influence on the rheological properties of chocolate (Franke et al., 2002). Examination of spray-dried milk powders produced by mixing milk fat fractions into skim milk prior to drying, spraying milk fat fractions onto dried powder or a combination of these showed that there was a good correlation between free fat content of the spray-dried powders and viscosity of chocolate mass, although other factors such as the microstructure and interfacial properties of the powders also had a role (Attaie et al., 2003).

MILK POWDERS WITH HEALTH PROMOTING FUNCTIONAL INGREDIENTS

The interest in the development of health promoting foods has led to research in functional milk powders for health and well-being. These include milk powders enriched with well-known nutrients such as minerals (e.g., Ca) and vitamins (e.g., vitamins A and D) and functional ingredients of more recent interest such as omega-3 oils, probiotics, and phytosterols. Some of these functional ingredients are added as microencapsulated ingredients while others are directly incorporated into milk powders (Augustin, 2003).

The well established role of calcium in bone health has driven interest in the development of calcium fortified milk products (Augustin and Williams, 2002). Insoluble calcium salts may be dry blended with milk powders but there are potential problems with separation during powder storage and settling of these salts when used in reconstituted milk applications. Soluble calcium salts may be added but their addition

has to be carefully managed to avoid protein precipitation of milk during heating as the direct addition of soluble calcium salts increases calcium activity and reduces the pH of the milk, making it more susceptible to coagulation. A strategy based on the addition of soluble calcium salts in combination with orthophosphates for management of calcium activity and pH control has been applied for the production of calcium fortified milk powders with up to 8 g additional calcium per kg powder. This approach involves fortification of milk followed by a low or high heat treatment of milk prior to concentration and drying (Williams et al., 2005).

Probiotics have a role in gut health and have been added to a range of foods. They are generally supplied as freeze-dried cultures for addition to foods. It is possible to produce a probiotic milk powder by spray drying reconstituted skim milk containing *L. paracases* NFBC 338 with a probiotic survival of 85%. It was further demonstrated that the probiotic powder could be added to cheese milk for production of probiotic cheddar cheese (Gardiner et al., 2002).

The incorporation into foods of omega-3 fatty acids has been increasing due to their link with improved heart, eye, and brain function. Spray-dried milk powder enriched with omega-3 oils may be produced from full-cream milk supplemented with a range of omega-3 oils (e.g., fish oil) prior to spray drying. Special care needs to be taken in the production of omega-3 enriched milk powders because omega-3 oils are susceptible to oxidative deterioration. Omega-3 enriched milk powders containing 2.4 and 2.1% eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively, that were made by supplementation of milk with fish oils were found to be stable for ~5 months (Ramaprasad et al., 2006). An alternative process is to dry blend stabilized microencapsulated omega-3 powders with milk powders, an approach that has been used for the production of omega-3 enriched infant formulae currently available in the market.

Phytosterols are added to a variety of foods because of their cholesterol-lowering properties. A potential limiting factor for the use of these compounds in food is their susceptibility to oxidation which leads to the formation of undesirable by-products. However, this was not an issue for a phytosterol-enriched whole milk powder containing 7% phytosterol. A phytosterol-enriched milk powder, which has been produced by spray drying a concentrated milk emulsion with incorporated microcrystalline phytosterol suspension in fat, was stable for 12 months at room

temperature or slightly elevated temperatures of 38°C (Soupas et al., 2006).

CONCLUSION

Conventional skim and full-cream milk powder products are expected to remain major commodities of the dairy industry. However, the market demands for milk-based powders with enhanced functionality for specific end-uses with more stringent functionality requirements will continue to drive the development of differentiated milk powders. The capacity of milk and dairy products to contribute nutritional and physical attributes to food products as well as have a physiological functional role will no doubt insure the long-term viability of the milk powder industry. The development of new dairy-based powders relies on continued research into the structure and function of milk components in various environments and how their properties can be manipulated and controlled by the application of conventional and emerging food processing technologies (Augustin and Udabage, 2007).

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15

Whey and Whey Products

Arun Kilara

Introduction

Basic Whey Products

Whey Powder

Partially Delactosed Whey Powder

Demineralized Whey Powder

Basic Whey Protein Products

Whey Protein Concentrate 35 (WPC 35)

Whey Protein Concentrate 50 (WPC 50)

Whey Protein Concentrate 80 (WPC 80)

Whey Protein Isolate

Permeate Processing

The Structure of Whey Proteins

β -Lactoglobulin

α -Lactalbumin

Bovine Serum Albumin

Immunoglobulins

Protease Peptones

Use of Whey Proteins as Ingredients

Water-Protein Interactions

Foaming

Emulsification

Gelation

Analytical Methods

References

INTRODUCTION

Whey is a product obtained from milk after removal of fat and casein. It is often a by product of casein or cheese manufacture. The major constituents of whey are lactose, proteins, minerals, and water. Water is the most abundant constituent in whey accounting for up to 95% by weight. The next abundant constituent is lactose followed by minerals.

Acid cheese or acid casein manufacture results in acid whey. In the United States, acid whey by law cannot be neutralized. In other countries such a law may

not exist. Whey resulting from process other than acid is termed sweet whey and constitutes the majority of whey produced in the United States. Whey is converted to various products such as condensed whey, dried whey, lactose, protein concentrates, and protein isolates. Collectively, these are referred to as whey products. Whey protein concentrates and isolates are value-added products and are rapidly moving toward commodity status. Further fractionation of whey proteins is developing as the next level of value-added products. The amounts of these whey products produced in the United States, in 2006, are listed in Table 15.1.

The composition of the whey is a variable because of the process changes in manufacturing the main product, either casein or cheese. Any variations in the manufacturing processes of the primary products could potentially alter the constituents that end up in whey. In general, the composition of whey from different sources is shown in Table 15.2.

Upon casual inspection of the composition of whey from different sources, the variations seem to be small to negligible. However, in the manufacture of value-added products the seemingly minute differences are magnified many fold. Ash or the mineral components of acid and sweet whey are shown in Table 15.3.

Increased calcium content of acid whey is the direct result of the bound calcium in the casein micelles being released as acidification progresses. The approximate ratio of free to bound calcium in native casein micelles is 1:3. The lactate content is also high as a result of lactic acid being produced during the acidification process. The lactate content of sulfuric acid casein is much lower because no fermentation is involved. Phosphate content of acid whey is somewhat

Table 15.1. Production of Whey and Modified Whey Products in the United States in 2005 (USDA) in Metric Tones

Product	Quantity (Mt)
Condensed whey solids	53,460
Dry whey	550,173
Human use	524,570
Animal feed	23,308
Lactose and mineral reduced whey solids	45,789
Lactose	369,328
Whey protein concentrate (WPC)	213,862
Human use	189,309
Animal use	24,553
WPC 25–50% protein	148,733
WPC 50–90% protein	65,129
Whey protein isolate (>90% protein)	15,337

higher than sweet whey as is the magnesium content. Calcium, magnesium, and phosphate are involved in forming salt bridges in the native casein micelles. These are disrupted by acidification and become unbound from the casein micelle.

The physicochemical properties of whey vary with the source of the whey and when compared with skim milk have less viscosity and a lower surface tension. The freezing point is lower than skim milk and under heating conditions the proteins are unstable. Whey proteins are however stable changes in pH.

The major proteins in whey are β -lactoglobulin (β -lg), α -lactalbumin (α -la), bovine serum albumin (BSA) immunoglobulins (Ig). There are also non-protein nitrogen components present. By definition whey proteins are soluble when pH of milk is adjusted to 4.6. The abundance of these proteins and some of their properties are listed in Table 15.4.

Table 15.3. Mineral Composition of Acid and Sweet Whey

Constituent	Concentration (g/L)	
	Sweet Whey (pH 5.9–6.4)	Acid Whey (pH 4.6–4.8)
Calcium	0.04–0.06	1.2–1.6
Magnesium	0.08	0.11
Phosphate	1.0–3.0	2.0–4.5
Citrate	1.2–1.7	0.2–1.0
Lactate	2.0	6.0
Sodium	0.4–0.5	0.4–0.5
Potassium	1.4–1.6	1.4–1.6
Chloride	1.0–1.2	1.0–1.2

These properties have an influence on the functionality of whey products in food systems and will be discussed later in this chapter.

On the basis of the value chain analysis whey products can be classified as basic products (whey powder and its variants), value-added products (whey protein concentrates and isolate), and specialized products (lactoperoxidase, lactoferrin, whey protein peptides, and nutraceuticals).

BASIC WHEY PRODUCTS

WHEY POWDER

The basic whey products are whey powder, partially demineralized, partially delactosed, partially demineralized, and demineralized whey powders. In all whey processing, there are a few preprocessing steps. These steps prepare the substrate (whey) for further processing.

There are fine particles of curd that are drained with the whey. The amount and size of these particles depend upon the variety of cheese being manufactured

Table 15.2. Percent Composition of Cow's Whey from Different Commercial Processes

Constituent	Casein Whey		Mixed Whey	Sweet Whey	Acid Whey
	Rennet	Lactic			
Dry matter	7.08	6.58	7.05	7.00	6.50
Lipids	0.51	0.09	0.34	0.20	0.04
Lactose	5.18	4.53	5.05	4.90	4.40
Total nitrogen	0.15	0.12	0.15	0.13	0.11
Acids (lactic and citric)	0.16	0.78	0.32	0.20	0.05
Ash	0.53	0.07	0.47	0.50	0.80

Table 15.4. Constituents of Whey Proteins and Some Important Physicochemical Properties

Protein	Abundant (g/L)	Total Whey Protein (%)	Molecular Weight	Disulfid Bonds/mol	pI	Denatured Temperature (C)
α -lg	4.0	50	18,362	2	5.2	82
β -la	1.5	19	14,174	4	4.5–4.8	61
BSA	0.4	5	69,000	17	4.7–4.9	66
Ig	1.0	13	150,000–1,000,000	—	5.5–8.3	72

and other processing variables. These fine are separated by vibrating sieves, centrifugal separators, or by hydrocyclones. The recovered fine may either be recycled into the cheese or used in other foods like processed cheeses. Depending upon the type of cheese manufactured, free fat may also drain with the whey. After separation of the casein fine (curd fines) the whey is subjected to centrifugal separation to recover the fat. The recovered fat is converted to whey butter, an industrial ingredient. After these two processes, the whey is clear and translucent. The whey can then be cooled or pasteurized and cooled and stored for further processing.

At this stage, whey has approximately 5% total solids making it uneconomical to dry without further concentration. Concentration may be achieved by vacuum evaporation or by reverse osmosis. Because concentration by reverse osmosis alone is very expensive and the total solids achieved by such concentration are still not high enough it seldom used as a stand alone process. Instead, it is used in conjunction with vacuum evaporation. The final concentration of total solids after evaporation should be 45–65%. The upper end of this range may pose some problems with hygroscopicity in the drier.

The concentrated whey is cooled rapidly to 30°C in a plate heat exchanger and the cooled whey concentrate is placed in a refrigerated tank fitted with an effective agitator where it is cooled to 15–20°C. Constant agitation is critical and the time for such repose can be 6–8 hours. During this time lactose crystallizes to the smallest size possible. Such crystallization assures nonhygroscopicity of the powder made from this concentrate. Concentrated whey is a super saturated solution of lactose and this sugar is prone to crystallization. Under certain conditions (high solids), the lactose may crystallize in the evaporator which makes the concentrate so thick that it can no longer flow. This situation is to be avoided rigorously.

The drying of whey concentrate is achieved by the same processes used for drying milk. Generally, drum

drying is not used for whey drying because it causes burn on and scorched particles. This occurs because the film of dry whey is very hard to scrape off the drum. Spray drying is the process of choice.

PARTIALLY DELACTOSED WHEY POWDER

This product is also called reduced lactose whey. Modification of lactose content is achieved by one of two methods. Lactose can be removed by selective removal of lactose or alternately lactose can be hydrolyzed into its constituent sugars by the enzyme β -galactosidase (lactase).

Selective removal of lactose can be achieved by removing lactose crystals after the whey concentrate is cooled and seeded. In order to do this the total solids concentration of the whey concentrate has to be in excess of 65%. Such a high solids concentration may pose problems in the evaporator but can be achieved by careful controlled operation of the evaporator. Another method is to use ultrafiltration and diafiltration to remove lactose. However, ultrafiltration will also remove minerals, nitrogenous molecules along with lactose, and concentrate the protein in the retentate. Breakdown of lactose by enzyme can result in varying degrees of hydrolysis by controlling the contact of the enzyme with the substrate. Termination of the enzyme reaction is achieved by heating the substrate or pasteurizing the concentrate. Reduced lactose powders contain 18–24% protein, 52–58% lactose, 1–4% fat, 11–22% ash, and 3–4% moisture.

DEMINERALIZED WHEY POWDER

This product is also called reduced-minerals whey powder. To manufacture this product, a portion of the minerals in whey is removed prior to concentration and drying. Various degrees of demineralization can be achieved with 25, 50, or 90% of the minerals being removed. Minerals are removed by ion exchange, nanofiltration or electrodialysis.

Ion Exchange Process

Ion exchange processes have three steps: adsorption, desorption, and regeneration. Both cationic and anionic resins are required for the demineralization of whey. Strongly acidic gel-type styrene polymeric resins are used for cation adsorption and removal. Medium basic gel-type styrene polymers or acrylic polymers are used for anion exchange. Whey is demineralized by replacing the mineral ions in the cationic columns with hydrogen ions and then in the anionic columns the hydrogen ion is replaced with the hydroxyl ion. When the resins become saturated with ions, they are regenerated by removal of adsorbed ions with hydrogen ions in the cationic exchanger and by removing the adsorbed hydrogen ions in the anionic and replacing it with hydroxyl ions. While demineralization of whey is done in a co-current flow through the resin beds, regeneration is done by counter-current flow of the regenerants in the columns. For the cationic column, regeneration is achieved by the use of dilute sulfuric acid solution. Care must be taken to avoid the formation of insoluble calcium phosphate. Anionic columns are regenerated using dilute caustic solutions. Prior to regeneration the resin beds are flushed with water to recover the demineralized whey. The flow rate of the water should be equal to the flow rate of the whey. These steps are time-consuming and therefore only 3 cycles per day are possible with concentrated whey and 4 cycles per day with native whey. A cycle is defined as the completion of adsorption, followed by whey recovery and then regeneration. Typically for both concentrated and native whey, it takes 1.5 hours for the demineralization step. The regeneration step takes 4.5 hours with native whey and 5.5 hours with concentrated whey. This totals to a 6-hour cycle time for native whey and a 7-hour cycle time for concentrated whey. Therefore, a maximum of 4 batches of native whey and 3 batches of concentrated whey can be processed in a 24-hour period. These examples are for a single line. However, multiple lines are generally used to increase processing capacity and these operations are fully automated.

To process 500,000 kg of native whey per day approximately 16,000 liters of cation exchange resin and 10,800 liters of anionic resin would be required. If the whey was preconcentrated by nanofiltration and 130,000 kg of concentrated whey is processed 13,700 liters of cationic resin and 9,000 liters of anionic resin would be required. In the regeneration

process approximately 250 kg of sulfuric acid and 120 kg of caustic would be used per ton of dry matter processed. If the whey was concentrated by nanofiltration then 165 kg of sulfuric acid and 75 kg of caustic would be required. Ion exchange processes can achieve 92–97% demineralization.

There are a number of variables in calculating these values. Ion exchange capacity of the resin, swelling properties, mechanical strength, fluidization during back flushing pressure drop, flow velocity restrictions, and water rinse requirements after regeneration are all important variables in designing ion exchange processes. Approximately, 10–15 bed volumes of whey can be treated per regeneration and a larger volume of resin is required for cation exchange than for anion exchange.

Nanofiltration

Nanofiltration is a membrane process. This process concentrates proteins and organic materials by removing water and some salts (particularly monovalent ions). This process is used with native whey and/or with ultrafiltration permeates. *Permeate* is the term used for materials passing through a membrane (filtrate) while the residue is called retentate. Nanofiltration is a cross-flow membrane separation process. Typically, the nanofiltration membrane is spirally wound. The separation mechanisms of nanofiltration consist of steric and electrical effects. Nanofiltration of a multicomponent mixture, like whey, is a complex process with numerous interactions. Nanofiltration membranes do not have visible pores but they have some free volume depending on their openness and structure. Pore in a nanofiltration membrane is a polymer material free void space through which fluids can be transported under a driving force. The most common driving force is pressure. The electrical effects concern the Donnan equilibrium. When osmotic equilibrium between two electrolyte solutions, one of which contains ions to which the membrane is impermeable, fluid depends not only on pressure differences but also differences in electrical potentials across the membrane. This allows for less charged molecules to go across the membrane while retaining the more charged ones. In whey, divalent ions are retained while monovalent ions traverse the membrane. The separation characteristics of nanofiltration are intermediate between reverse osmosis and ultrafiltration. The pressures in a nanofiltration process range from 0.6 to 4 MPa.

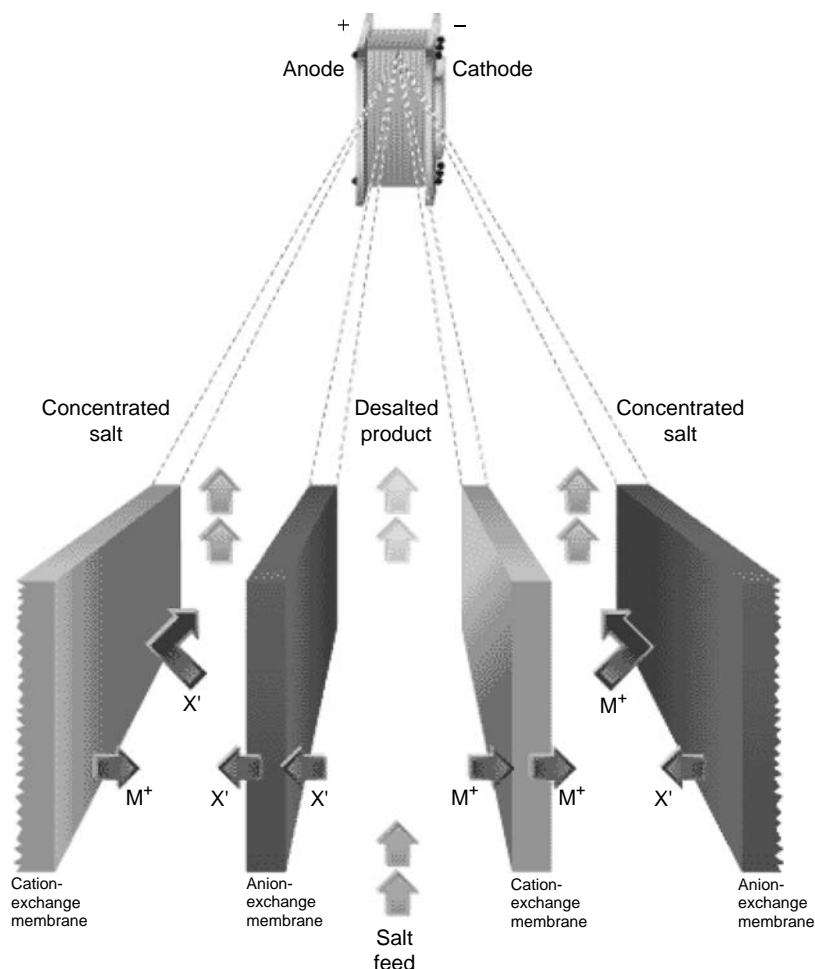


Figure 15.1. Schematic of an electrodialysis stack.

The total solids of whey are 5–6% and by the nanofiltration process it increases to 15–25%. The total mineral content is concomitantly reduced to obtain 40–50% demineralization. This amounts to a 3- to 5-fold concentration. Higher total solids concentrations increase the risk of calcium phosphate precipitation which cause membrane fouling and drastically reduce flow rates.

Electrodialysis

This process occurs when ions are transported across a nonselective semipermeable membrane in which the driving force is an applied potential generated by direct current. The membranes used can be

anionic or cationic. Thus, control over the types of ions removed is exercised. A number of compartments are separated by alternating cation and anion exchange membranes set 1 mm or less apart. The electrodes act as bookends to this assembly and as many as 200 compartments can separate the two ends (Fig. 15.1).

Anions can pass through an anion exchange membrane but are stopped by a cation membrane. Conversely, the cations pass through a cation exchange membrane but are stopped by the anion membrane. This is how the whey cells are depleted of ions. The degree of demineralization is dependent upon the ash content of the whey, current density, viscosity, and the residence time in the equipment. In a batch

electrodialysis system, in which whey is recirculated over membrane stacks repeatedly until the desired degree of demineralization is achieved involves temperatures of 30–40°C and periods of 5–6 hours. In order to make the process energy efficient whey should be concentrated to 20–30% solids prior to batch electrodialysis. The high temperature and long time increase safety concerns from a microbiological point of view. This risk can be controlled by the addition of bacteriostatic agents such as hydrogen peroxide and by adding a cooling step to remove the heat added due to the electrical current.

In a continuous process of electrodialysis the process time is reduced to 10–40 minutes and only 60–70% demineralization is achieved. For certain applications such as infant food formula 90% demineralization is specified. Additional demineralization is conducted in a batch mode to obtain the desired specifications. Alternately, the whey demineralized by a continuous process can be subjected to ion exchange process. Units are cleaned in place and this process takes approximately 1.5–2 hours. Power requirements for electrodialysis has to be varied over the process from 0 to 185 A and 0–400 V and it is direct current.

Some disadvantages of the electrodialysis process include the precipitation of calcium phosphate on the cation membrane surfaces (mineral fouling) and the deposition of protein on the anion exchange membranes (protein fouling). The replacement costs of membranes are 30–40% of the total running costs of the process. Mineral fouling is minimized by proper flow design over surfaces and by regular acid cleaning. Protein fouling is more important cause of membrane failure than mineral fouling. The whey proteins exist as molecules with a predominantly negative charge and move as anions in the electrical stack. Proteins being macromolecules do not pass through the anion membranes and are deposited as a thin layer on anion membranes. Techniques such as polarization reversal can help remove proteins from the membranes. High pH (alkaline) cleaners also remove protein residues but can damage the membranes. Hand cleaning of such anion membranes is recommended at intervals of 3–4 weeks. Electrodialysis is best suited to achieve demineralization of 70% or less. Higher levels of demineralization are not cost effective when compared to ion exchange.

The combination of processes involving removal of lactose and minerals results in partially delactosed and partially demineralized whey powder.

Table 15.5. Proximate Composition of Whey Protein Products

Constituent	Whey Protein Concentrate Protein (%)		
	35	50	80
Moisture	4.6	4.3	4.0
Crude protein	36.2	52.1	81.0
True protein	29.7	40.9	75.0
Lactose	46.5	30.9	3.5
Fat	2.1	3.7	7.2
Ash	7.8	6.4	3.1

BASIC WHEY PROTEIN PRODUCTS

The category called basic protein products includes whey protein concentrate containing 34, 55, or 75–80% protein on a moisture-free basis. These protein products have achieved commodity status that is, they are main stream ingredients sold at competitive prices and profit margins are diminishing year by year. When milk prices increase and milk is in short supply, the prices of whey protein products increase as well and follow the economic laws of demand and supply. In the years 2006–2008, whey protein prices fetched premium prices due to a global shortage of milk and milk products. The proximate composition of whey protein products is shown (Table 15.5).

As the protein content increases the fat content increases and lactose, moisture, and ash contents decrease. The process commonly used for protein concentration is ultrafiltration a membrane-based process. The membranes not only retain the protein but also the fat and thus their relative abundance increases. Similarly, in this process lactose and minerals are lost in the permeate hence their decrease as a percentage of the remaining solids.

WHEY PROTEIN CONCENTRATE 35 (WPC 35)

This ingredient is used as a substitute for nonfat dry milk (NFDM) or skim milk powder. The ash content and mineral profile are different than NFDM.

Whey that is clarified and defatted is subjected to ultrafiltration. The total solids of the permeate consists mainly of lactose and some minerals. The total solids of the retentate consist in almost equal parts of lactose and protein and some minerals. This is

then concentrated by vacuum evaporation to 55% total solids and spray dried.

WHEY PROTEIN CONCENTRATE 50 (WPC 50)

The first steps are similar to the manufacture of WPC 35. Pasteurized sweet whey is subjected to ultrafiltration to obtain permeate and retentate streams. The retentate is subjected to a washing procedure called diafiltration in which water is added in a volume equal to that of the retentate. The diluted retentate is passed through the ultrafiltration membranes. Total solids of the diafiltration permeate consist predominantly of lactose and some minerals. The total solids of the diafiltration retentate are made up predominantly of protein followed by lactose and minerals. The retentate stream is then subjected to vacuum evaporation to obtain about 50–55% total solids prior to spray drying.

WHEY PROTEIN CONCENTRATE 80 (WPC 80)

The manufacture of WPC 80 consists of increased washing of the retentate obtained from the process of WPC 50 manufacture. Instead of washing the retentate with 1 volume of water the diafiltration step involves 2–3 volumes of water. The additional volumes of water result in washing more lactose and minerals into the permeate stream thereby increasing the protein concentration. A general schematic is shown in Figure 15.2.

WHEY PROTEIN ISOLATE

By definition isolates are protein products that contain >90% protein on a moisture-free basis. Thus, whey protein isolate (WPI) is a protein product that contains >90% protein.

Two methods are commonly used to manufacture WPI. The older of the two methods involves ion exchange process while the newer method relies on membrane-based fractionation techniques. Proteins are amphoteric macromolecules, that is, they carry both positive and negative charges. The net charge on a protein molecule is dependent on the amino composition and the environmental pH. Under acidic conditions proteins assume a net positive charge (cationic) and at neutral or slightly alkaline pH values most proteins are negatively charged (anionic). Isoelectric

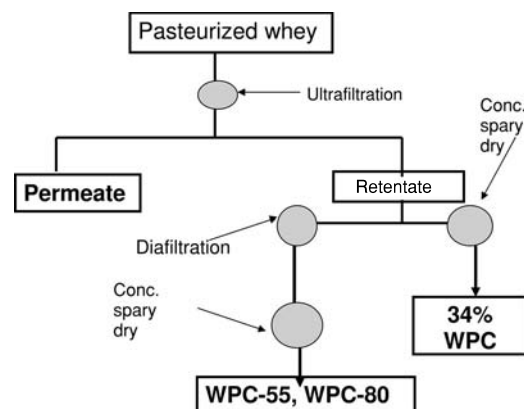


Figure 15.2. Schematic of process for the manufacture of whey protein concentrates.

point is the pH at which there are an equal number of positive and negative charges.

Adsorption of proteins on an ion exchange process can be achieved in a stirred tank reactor in which carboxymethyl cellulose is the ion exchange matrix. The pH of the whey is adjusted to 3.2. The desorption is accomplished by changing the pH to near neutral (pH 7–7.5). The dilute eluate containing the protein is filtered, concentrated by membrane processes, further concentrated by vacuum evaporation and then spray dried. This process can yield 97% protein on a dry weight basis and 3% ash (minerals) with only traces of lactose and fat (approximately 0.2% each).

Since stirred tank reactors are not efficient a newer process was designed using resin packed in a column. The Spherosil Process uses either cationic or anionic resins.

Spherosil resins consist of silica beads coated with a polymer containing the appropriate functional exchange group. The cation exchanger has $-\text{SO}_3\text{H}$ groups and is called Spherosil-S. The anion reactive group is $-\text{N}(\text{CH}_3)_3$ and is called Spherosil QMA. Using Spherosil-S whey is acidified to pH 4.5 to facilitate adsorption of protein. Elution is performed with ammonium hydroxide. If Spherosil QMA is used, sweet whey at pH 6.3 is used for adsorption while elution utilizes hydrochloric acid.

Unlike carboxymethyl cellulose, Spherosil does not swell with changes in pH or ionic strength, can withstand pressure and is physically and chemically stable. However, it is expensive and has a low protein binding capacity (79 mg/g) compared to CMC

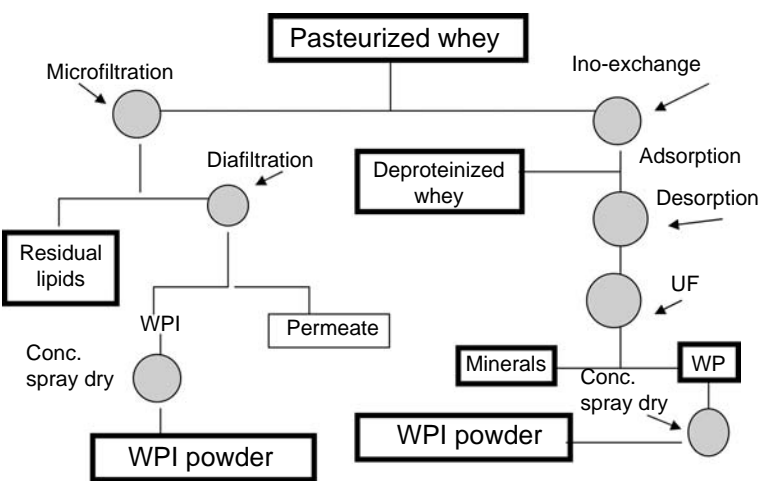


Figure 15.3. Schematic for process to manufacture whey protein isolate.

(200–500 mg/g). The recovery and yield is lower with anion exchange resin (Spherosil QMA) because basic whey proteins such as lactoferrin and lactoperoxidase are not adsorbed. In addition, Ig are also not adsorbed by the anionic resins.

The other process to obtain whey protein isolate is by using membrane processing. Sweet whey is microfiltered to recover residual fat. Fat impacts functional properties of whey proteins, especially foaming properties. The delipidated sweet whey is subjected to ultrafiltration and extensive diafiltration to obtain whey protein isolate solution which is concentrated and spray dried. This membrane processing method of whey protein isolate manufacture drastically lowered the cost of this ingredient (Fig. 15.3).

PERMEATE PROCESSING

Permeate generated through ultrafiltration and ultrafiltration–diafiltration contains lactose and some minerals. Permeate is concentrated either by reverse osmosis, vacuum evaporation, or a combination of the two processes. Lactose and minerals are concentrated by these water removal processes.

Permeate is used as animal feed supplying calories and minerals or is further processed to extract lactose and isolate the milk salts.

The main products obtained by permeate processing are dried dairy solids, lactose, lactose-reduced dairy solids, dairy minerals.

Permeate powder also named dairy products solids is concentrated permeate in which lactose has been

crystallized and is followed by the drying of the concentrate (Fig. 15.4).

This product serves as a direct replacement of other dairy solids in applications such as bakery and confectionery products. Because it is a concentrated source of lactose, it produces brown crusts in bread and other baked goods. It is also fermentation medium for the production of bacteriocins.

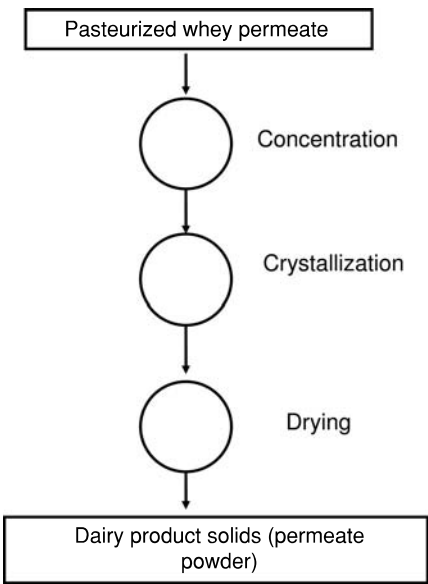


Figure 15.4. Schematic for processing permeate.

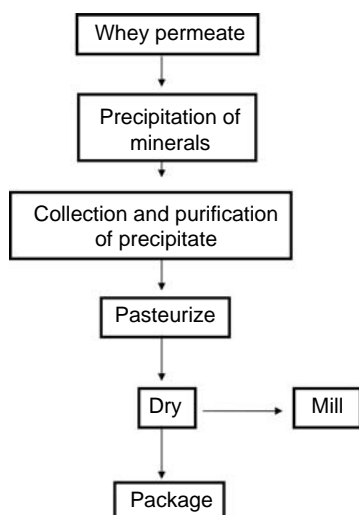


Figure 15.5. Schematic for processing milk minerals.

Mineral-concentrated whey, also called reduced lactose whey, is a product manufactured by partial removal of lactose and is used as a functional ingredient in meat, confectionery, bakery, snacks, seasonings, soups, sauces, and gravies and dry mixes.

Dairy minerals, calcium are derived from whey permeate from which minerals are precipitated (Fig. 15.5).

The minerals are then collected and purified by reprecipitation followed by pasteurization and drying.

It may also be milled prior to packaging. This type of product contains 23–28% calcium and 13–14% phosphorous. It is typically used in nutritional supplements, bars, and chews, calcium-fortified foods such as baked goods, processed meats, dairy and confectionery products. It is also used in calcium-fortified beverages such as juices and dairy drinks.

Lactose, the most abundant component of milk solids is manufactured from pasteurized whey or permeates. The fluid is concentrated and the lactose is crystallized (Fig. 15.6).

Crystals are recovered by centrifugation of the mother liquor. Centrifugation results in two streams, the first being partially delactosed whey or permeate (depending on the starting material) and lactose crystals. Lactose crystals are refined and dried.

In summary, the range of commercial whey products available are whey powders (sweet type, acid type), reduced lactose whey powder, whey protein concentrates (WPC) with protein contents varying between 34 and 80%, whey protein isolate, deproteinized whey, whey permeate, milk calcium minerals, and lactose (food grade and pharmaceutical grade).

THE STRUCTURE OF WHEY PROTEINS

β -LACTOGLOBULIN

The most prevalent protein in whey is β -lactoglobulin. It comprises 10% of the total milk

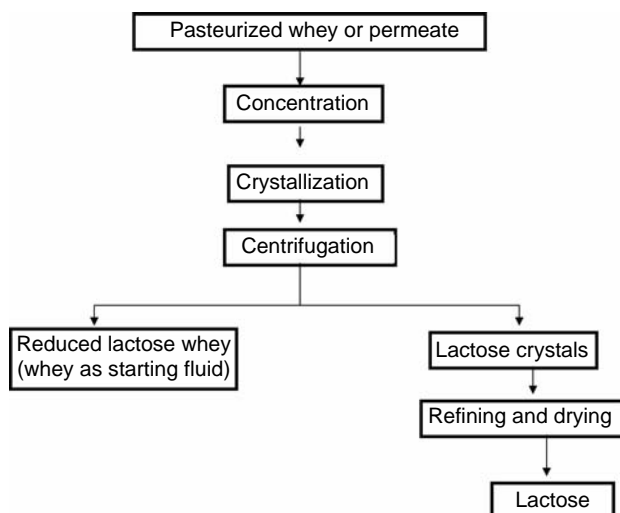


Figure 15.6. Schematic for processing of lactose.

1	Leu	Ile	Val	Thr	Gln	Thr	Met	Lys	Gly	Leu	11	Asp	Ile	Gln	Lys	Val	Ala	Gly	Thr	Thr	Trp
21	Ser	Leu	Ala	Met	Ala	Ala	Ser	Asp	Ile	Ser	31	Leu	Leu	Asp	Ala	Gln	Ser	Ala	Pro	Leu	Arg
41	Gln in Variant D										51	Variant C His									
Val	Tyr	Val	Glu	Glu	Leu	Lys	Pro	Thr	Pro	Glu	Gly	Asp	Leu	Glu	Ile	Leu	Leu	Gln	Lys		
61	Gly in Variants B, C										71										
Asp	Glu	Asn	Asp	Glu	Cys	Ala	Gln	Lys	Lys	Ile	Ile	Ala	Glu	Lys	Thr	Lys	Ile	Pro	Ala		
81											91										
Val	Phe	Lys	Ile	Asp	Ala	Leu	Asn	Glu	Asn	Lys	Val	Leu	Val	Leu	Asp	Thr	Asp	Tyr	Lys		
101											111	Variants B, C Ala									
Lys	Thr	Leu	Leu	Phe	Cys	Met	Glu	Asn	Ser	Ala	Glu	Pro	Glu	Gln	Ser	Leu	Val	Cys	Gln		
121											131										
Cys	Leu	Val	Arg	Thr	Pro	Glu	Val	Asp	Asp	Glu	Ala	Leu	Glu	Lys	Phe	Asp	Lys	Ala	Leu		
141											151										
Lys	Ala	Leu	Pro	Met	His	Ile	Agr	Leu	Ser	Phe	Asn	Pro	Thr	Gln	Leu	Glu	Glu	Gln	Cys		
161 162																					
His	Ile	OH																			

Figure 15.7. Primary structure of bovine β -lactoglobulin A. The locations of the amino acid substitutions in the genetic variants are indicated. There is a disulfide bond between cys 66 and cys 160. Another disulfide bond is formed between cys119 and cys 121. There is a 50:50 distribution of the bond between positions 119 and 121. Cys 121 is always involved in the bond.

protein or about 58% of the whey protein. It contains 162 amino acids with a molecular weight of about 18,300. There are two genetic variants, A and B that differ in the substitution of a glycine in Variant B and an aspartic in Variant A. The molecule contains two disulfid and one free sulfhydryl groups and no phosphorus (Swaigood, 1982).

The primary sequence of β -lactoglobulin (Fig. 15.7) shows one of the disulfid groups between cys 66 and 160.

The other seems to be a dynamic one that involves 106 and is sometimes found with cys 121 and sometimes with cys 119. Thus, 1/2 of the cys 119 and 1/2 of the cys 121 exist as free sulfhydryl groups (Kinsella, 1984).

Below pH 3.0 and above pH 8.0, β -lactoglobulin exists as a monomer. Between pH 3.1 and 5.1 at low temperatures and high protein contents, it associates to form an octamer. This polymerization seems to be mediated through the action of carboxyl groups and thus the Variant A forms better octamers than does the Variant B. At other pH values, including the pH of milk, β -lactoglobulin tends to be found as a dimer. These dimers are spherical with diameters of about 18 Å. The complex association–dissociation behavior of β -lactoglobulin has been the subject of extensive study (Whitney, 1977).

β -Lactoglobulin is manufactured specificall in the mammary gland for inclusion in milk where

its role is unknown. All ruminant milk contains β -lactoglobulin while the milk of almost all non-ruminants does not. Biological functions have been speculated to exist for β -lactoglobulin, to date none have been fully accepted. The molecule has a very hydrophobic area that is quite effective in binding retinol. Some speculate that the binding of vitamin A may have a regulatory role in the mammary gland. Because of its prevalence in bovine milk, to a large extent the properties of whey protein concentrates are in effect with respect to the properties of β -lactoglobulin.

The secondary structure of β -lactoglobulin is homologous to that of retinol-binding proteins. It contains 9 strands of β structure, 8 of them arranged to form a β barrel. The lone α helix is located on the surface of the molecule. The center of the barrel is hydrophobic and can be involved in the binding of hydrophobic molecules. The three-dimensional structure of β -lactoglobulin is (Fig. 15.8) similar to plasma retinol-binding protein (Papiz et al., 1986).

α -LACTALBUMIN

The second most prevalent protein in whey is α -lactalbumin that comprises about 2% of the total milk protein that is about 13% of the total whey protein. The molecule consists of 123 amino acids and has a molecular weight of 14,146. The molecule

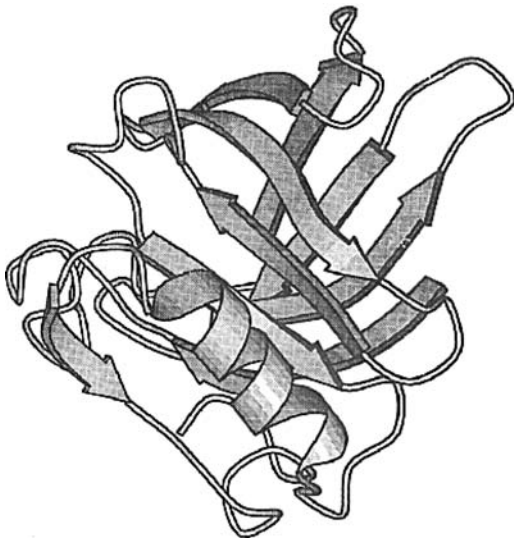


Figure 15.8. Structure of bovine β -lactoglobulin.

contains four disulfid linkages and no phosphate groups. Its primary structure (Fig. 15.9) has been elucidated (Brew et al., 1970).

The three-dimensional structure for α -lactalbumin from baboon is also shown (Fig. 15.10).

α -Lactalbumin has been shown to modify the activity of the enzyme galactosyl transferase. In the absence of α -lactalbumin, this enzyme adds UDP-galactose to N-acetyl glucosamine groups that are attached to proteins. It can transfer the UDP galactose

to glucose, but the K_m for glucose is 1,400 mM and thus, the reaction proceeds slowly, if at all. α -Lactalbumin serves to lower the K_m for glucose to 5 mM and the enzyme complex now will add UDP-galactose to glucose to produce lactose and UDP. Thus, the milk of all mammals that contain lactose also contains α -lactalbumin. The α -lactalbumin of any species isolated so far will serve to modify bovine galactosyl transferase activity (Brew and Grobler, 1990).

When the sequences of α -lactalbumin and lysozyme are compared, about 40% of the residues are found to be the same, including all the cysteine residues. Another 20% of the residues have similar structures. This information coupled with the fact that α -lactalbumin helps to synthesize the same linkage that lysozyme cleaves, suggests that the molecules are closely related. In fact, knowledge of the three-dimensional structure of lysozyme has been utilized to predict the three-dimensional structure of α -lactalbumin (Browne et al., 1969).

Despite their similarity, they do not work on the same substrates and are not related antigenically. The site of synthesis of α -lactalbumin like β -lactoglobulin is the mammary gland. α -Lactalbumin is unusual in that the molecule is more stable to heat in the presence rather than the absence of calcium. Most proteins show increased heat sensitivity in the presence of calcium. This is probably due to the ability of calcium to promote the formation of ionic intermolecular cross-links with most proteins. These crosslinks hold the molecules in proximity and increase the likelihood of aggregation upon heating.

1	Arg in Variant B																			
Glu	Gln	Leu	Thr	Lys	Cys	Glu	Val	Phe	Gln	Glu	Leu	Lys	Asp	Leu	Lys	Gly	Tyr	Gly	Gly	
21	31																			
Val	Ser	Leu	Pro	Glu	Trp	Val	Cys	Thr	Thr	Phe	His	Thr	Ser	Gly	Tyr	Asp	Thr	Glu	Ala	
41	51																			
Ile	Val	Glu	Asn	Asn	Gln	Ser	Thr	Asp	Tyr	Gly	Leu	Phe	Gln	Ile	Asn	Asn	Lys	Ile	Trp	
61	71																			
Cys	Lys	Asn	Asp	Gln	Asp	Pro	His	Ser	Ser	Asn	Ile	Cys	Asn	Ile	Ser	Cys	Asp	Lys	Thr	
81	91																			
Leu	Asn	Asn	Asp	Leu	Thr	Asn	Asn	Ile	Met	Cys	Val	Lys	Lys	Ile	Leu	Asp	Lys	Val	Gly	
101	111																			
Ile	Asn	Tyr	Trp	Leu	Ala	His	Lys	Ala	Leu	Cys	Ser	Glu	Lys	Leu	Asp	Gln	Trp	Leu	Cys	
121	123																			
Glu	Lys	Leu	OH																	

Figure 15.9. Primary structure of bovine α -lactalbumin B. The position of the amino acid substitution that occurs in genetic variant A is indicated. Disulfide bonds are formed between the following pairs of cys residues: 6 and 120, 28 and 111, 61 and 77, and 73 and 91.

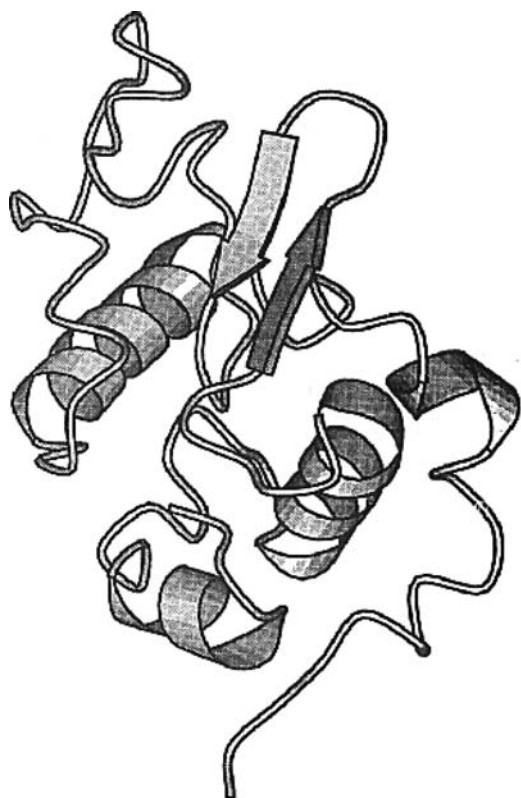


Figure 15.10. Three-dimensional structure of baboon α -lactalbumin (Bernstein et al., 1977).

α -Lactalbumin, on the other hand, uses calcium to form intramolecular ionic bonds that tend to make the molecule resistant to thermal unfolding. Under favorable conditions of calcium and pH, α -lactalbumin can remain soluble after exposure to 100°C. The structure of α -lactalbumin is presented in Figure 15.10 (Swaigood, 1996).

BOVINE SERUM ALBUMIN

The BSA, isolated from milk, is identical to the blood serum molecule. Thus, BSA is not synthesized in the mammary gland, but rather into the milk through passive leakage from the blood stream. The protein has a molecular weight of 69,000. It contains no phosphorus, 17 disulfides and 1 free sulfhydryl group. In blood plasma, albumin is a carrier of free fatty acids. The molecule has specific binding sites for hy-

drophobic molecules and may bind them in milk as well (Brown, 1977).

IMMUNOGLOBULINS

The Ig comprise at least 2% of the total milk protein. There are four classes of Ig found in milk: IgG1, IgG2, IgA, and IgM. All these molecules have a similar basic structure being composed of two light chains with molecular weights of 20,000–25,000 and two heavy chains, having molecular weights of 50,000–70,000 (Swaigood, 1982).

These molecules are not synthesized in the mammary gland and thus must first enter into the gland and then be transported through it to be able to enter the milk. In the case of at least one class of antibodies, IgG1, a specific receptor site has been located on the membrane of the cells of the mammary gland that facilitates the entry of this protein into the gland. The Ig supply passive immunity to the calf when supplied in the colostrum. This protection lasts until the animal is old enough to begin synthesis of its own antibodies (Whitney, 1977).

PROTEOSE PEPTONES

This fraction of milk has been defined as those proteins that remain in solution after milk has been heated at 95°C for 20 minutes and then acidified to pH 4.7 with 12% trichloroacetic acid (Swaigood, 1982).

This fraction can be divided into 4 major components while other minor components are recognized. Proteose peptone component 3 is found only in whey and is not associated with casein. This protein contains over 17% carbohydrate and has a molecular weight of 20,000. Antibody to proteose peptone component 3 will cross react with fat globule membrane and it has been suggested that this component is of membrane origin (Girardet and Linden, 1996).

Proteose peptone component 5 has a molecular weight of 13,000 and is associated with both the whey and casein fractions of milk. The molecule contains phosphorus and has been shown to consist of the N-terminal 107 amino acids of β -casein that arise from the proteolytic cleavage that yields the γ -caseins (Swaigood, 1982).

In a like manner, proteose peptone component, 8, fast, with a molecular weight of 3,900 represents the N-terminal 28 amino acids released from the cleavage of β -casein. The other major proteose peptone

component, 8 slow, has not yet been shown to be derived by the proteolysis of any milk proteins. In time, however, this will probably occur. The protein has a molecular weight of 9,900. As a group, the proteose peptones are by definition resistant to heating. They are also very surface-active due in part to their low molecular weights and also to the carbohydrate associated with component 3. About 1.1% of the total milk protein consists of proteose peptone. As some of these molecules are derived from the proteolysis of β -caseins, their concentration in any given milk can be expected to increase with time (Swaigood, 1982).

USE OF WHEY PROTEINS AS INGREDIENTS

The composition of whey products depends upon the methods employed to reduce lactose and ash contents. Morr and Foegeding (1990) and de Wit et al. (1986, 1988) studied samples of whey protein concentrates and isolates from various countries and observed differences in the proportions of individual whey proteins and also individual minerals in the ash. There can be several reasons for the observed differences and these include seasonal changes and lipid composition (Kilara, 1994).

Physicochemical attributes that make a protein useful in foods are called functional properties. Composition of whey protein products varies and a number of different types of whey protein products are available in the marketplace. Morr (1979) observes that for any food protein ingredient to be useful, it must be free from toxic and antinutritional factors, free of off-flavors and off-colors, compatible with other processes and ingredients in the formulation. Functional properties can be evaluated in model systems, model food systems, and in real foods. The complexity of evaluation increases from model systems to real foods. Further, functionality testing in model systems has not been standardized. Lack of standardization presents challenges in evaluating and comparing results within the same laboratory and between laboratories. Many empirical methods exist for functionality testing.

WATER-PROTEIN INTERACTIONS

Macromolecules are not soluble in the same manner as small molecules are. However, the amino acid side chains in the proteins can interact with water and

proteins can be suspended in water. This property is often used as an indicator of whey protein denaturation. Protein solubility is a function of temperature, pH, presence of other ions, and the values obtained for solubility are highly dependent on the methods used to achieve the solubility (Kilara, 1984). Proteins are least soluble at their isoelectric point but whey proteins are soluble over a wide range of pH values. This property of whey proteins makes it desirable for use in beverages.

Increase in temperature generally results in increased solubility of low molecular weight solutes. For proteins, however, increasing temperatures can lead to denaturation and, in turn, a decrease in solubility. There is a positive correlation between solubility and enthalpy for denaturation of whey proteins (Kilara and Mangino, 1991). During ultrafiltration of whey the resulting retentates can be spray dried directly or a pasteurization treatment can be provided prior to spray drying. Pasteurization of retentates decreases the β -lactoglobulin content of the subsequent whey protein concentrate manufactured (Mangino et al., 1987).

Another related property is the interaction of proteins with water. This property leads to thickening or an increase in apparent viscosity. Hydrogen bonding, ion dipole, and dipole-dipole interactions are all important mechanisms for water-protein interactions. Physical forces such as adsorption are also important in increasing viscosity. Insoluble proteins bind a lot of water. Heat denatured whey protein (lactalbumin) absorbs more water than undenatured whey protein (Morr, 1989).

Viscosity which results from water-protein interactions have been discussed extensively by de Wit (1989). He observed that viscosity of a whey protein solution increases above 65°C and even greater increase occurs at temperatures greater than 85°C. Between 65 and 85°C, whey proteins denature and above 85°C denatured proteins aggregate leading to further viscosity increases.

Practical uses of whey protein concentrates in which water-protein interactions are utilized include yogurt drinks, hard pack ice cream, low-fat ice cream, nonfat ice cream, and soft serve ice cream, yogurt, and sour cream and coffee whiteners. In cheese sauces, low-fat cream soups, creamy salad dressings, refrigerated pasta and orange marmalade, viscosity, and the ability of whey proteins to bind water are useful. Nutritional beverages, meal replacement beverages, sports beverages, and protein fortified-citru

beverages also rely on the solubility of whey proteins for successful formulation.

FOAMING

Foams are the result of the behavior of proteins at air–water interfaces. Rapid diffusion of molecules to the interface followed by molecular rearrangement allows these film to entrap air. With whey proteins heating is a prerequisite for foaming (Devilbiss et al., 1975). This is suggestive of a partial denaturation of whey proteins resulting in molecular rearrangements conducive to rigid high viscosity surface films. This confirms the work of Reichert et al. (1974) who reported that heating whey protein concentrates to a temperature 55–60°C led to improvements in foaming properties of whey protein concentrates. Cooling whey protein solutions to below 4°C reduces foaming. It has been speculated that this temperature-dependent foaming may be due to the effects of heat on β -lactoglobulin (Haggett, 1976).

Hydrophobicity and sulphydryl content are predictors of foaming in whey protein concentrates derived from acid whey (Liao and Mangino, 1987). These observations are similar to those made with commercial whey protein concentrates (Peltonen-Shalaby and Mangino, 1986). It has also been reported that native confirmation of β -lactoglobulin strongly affected foaming performance (Kim et al., 1987). Pasteurization of retentates reduces foaming properties of the subsequently dried whey protein concentrates (Mangino et al., 1987). When 11 commercial protein concentrates were tested for their foaming properties, Morr and Foegeding (1990) found considerable variability. In some samples even though the foam volume was adequate the foams were not stable.

Foaming properties of whey proteins play an important role in baked goods and confectionery creams.

EMULSIFICATION

This pertains to the behavior of the protein at oil–water interfaces. Emulsions are formed when energy is applied to disperse one phase into another of two normally immiscible phases. If the dispersed phase is oil and the continuous phase is water an oil-in-water emulsion results. When the continuous phase is oil and the dispersed phase is water a water-in-oil emulsion results. Emulsions can be liquid, semi-solid, or solid. In addition to the work performed for dispersing the two phases an energy barrier is necessary to

prevent coalescence of the dispersed phase. This energy barrier is provided by surfactants (emulsifiers and proteins are macromolecular surfactants. Emulsions are metastable systems and four types of instability, namely creaming, flocculation coalescence, and phase inversion, may be observed. Proteins retard gravitational separation of phases (creaming) and are not as efficient as small molecular weight surfactants at stabilizing emulsions against coalescence.

Whey proteins are not extensively used as emulsifiers. In one study by Pearce and Kinsella (1978) the oil phase volume was maintained at a constant 25% and whey protein concentrations were increased from 0.5 to 5% in emulsions. It was observed that the oil droplet size decreased as the protein concentration increased (Pearce and Kinsella, 1978). It has been demonstrated that whey proteins adsorb at the interfaces at a slower rate than other proteins like β -casein (Tornberg and Hermansson, 1977). Factors affecting whey protein emulsions include pH and ionic strength. Around their isoelectric point (pI) whey proteins form poor unstable emulsions (de Wit, 1989). If the milk used for cheesemaking is pasteurized or if the whey resulting from the cheesemaking is pasteurized the emulsification properties of the whey proteins are not adversely affected (Mangino et al., 1987). Pasteurization of the retentate greatly diminished the emulsion capacity of the proteins. Mangino et al. (1987) therefore demonstrated that the effects of heat treatment on whey proteins in milk, whey, and retentates have differing effects on emulsification functionality.

Whey proteins do help in emulsification in infant formula, meal replacement beverages, soups and gravies, and coffee whiteners. They are used in conjunction with low molecular weight emulsifiers

GELATION

Under the right circumstances the balance between polymer–polymer and polymer–water interactions results in the formation of networks or structures known as gels. Gels are capable of holding large amounts of water and other nutrients within the network. Coagula are not gels and are incapable of holding large amounts of water. In the two-step process of gel formation, the first step involves the denaturation of the protein and the second step is a rearrangement of the denatured molecules leading to aggregation and network formation (Ferry, 1948). Foegeding and Haman (1992) have suggested a more detailed description of the thermal gelation of proteins.

Ions such as calcium, sodium, and magnesium affect gelation of whey proteins (Varunsatian et al., 1983). At pH > 8 the chloride salts of anions increased the rate of aggregation and calcium was the most effective cation. Divalent cations lowered the denaturation temperature of the proteins. Sodium chloride increased the denaturation temperature. Heat sensitivity of β -lactoglobulin was enhanced by the presence of calcium ions. Even though this study did not study gelation, some insights into one aspect of gelation can be gained by the results presented.

Alkane binding by proteins is a measure of the hydrophobicity of the protein. Calcium content and hydrophobicity are predictors of gel strength (Kohnhorst and Mangino, 1985). Mangino et al. (1987) demonstrated that pasteurizing milk used for cheesemaking affected the ability of whey protein to gel at pH 6.5 but not at 8.0. Heating retentates significantly reduced gel strength but pasteurization of whey did not significantly alter gel strength. It has been reported that whey protein isolates heated at 90°C for 15 minutes at pH 6.5–8.5 form reversible gels at protein concentrations of 9–10% (Rector et al., 1989, 1991). The melting temperature of gels at pH 8 ranged from 24.5 to 57.8°C. The maximum enthalpy of formation was –858 calories per mole of crosslinks and a maximum storage modulus of 240 dynes/cm² was obtained after holding for 7 hours at 8°C.

Rinn et al. (1990) reported that whey protein concentrates prepared by microfiltration through 0.6 μ m pores exhibited superior gels at 4 and 5% protein concentration. In comparison, conventionally prepared whey protein concentrates required 9% protein concentration to form nonpourable gels. When 11 commercial whey protein concentrates were tested for their abilities to gel, some did not form gels at all, some required high protein concentrations in order to gel. Addition of sodium chloride to solutions led to a decrease in the minimum concentration required for gelling (Morr and Foegeding, 1990).

Gelation is an important functionality that is useful in baked goods, processed meats, surimi, desserts, and sour cream applications. In many food products multiple functionalities are at play and it is difficult to specify the degree of importance of each property relative to its successful application in formulations.

ANALYTICAL METHODS

Determination of total protein can be accomplished by a number of different techniques. Some of these

include gravimetric, nitrogen determination, amino acid analysis, colorimetric methods, spectrophotometric, and fluorometric methods (Darbre, 1987). When dealing with mixtures of proteins like whey protein concentrates and isolates a mere determination of protein content is not as meaningful as knowing the proportion of individual components in the mixture. Thus, such an exercise requires the separation of components in a mixture followed by a determination of the concentration of the separated entities. Separation may be achieved by utilizing the ionic nature of proteins where ion exchange chromatography or electrophoresis is the technique of choice. Other techniques may rely on the separation of proteins based on their size or on their shape as in gel permeation or size exclusion chromatography. Proteins can also be separated on the basis of their polarity as in high performance liquid chromatography (HPLC; Holme and Peck, 1993). In these techniques, the identity of the separated components is arrived by a combination of prior knowledge of the nature of the mixture or by indirect comparison to known standards. For example, components separated via electrophoresis under dissociative conditions can, with the help of standards, reveal the molecular weight of the molecules but it cannot tell the analyst that the band is a certain protein.

Polyacrylamide gel electrophoresis of whey proteins was first performed to quantify the individual components (Darling and Butcher, 1965). The separation and staining procedures were standardized and during each electrophoresis run a standard protein solution of whey proteins was also separated and stained under the same conditions as the test materials. In this way the standard solutions were subjected to the same treatment as the test solutions. Densitometric scanning of the stained protein-containing gels followed by peak area determinations was carried out. By comparison with standard peak areas individual protein concentrations of the test samples were determined.

Pearce (1984) reported that an HPLC method could be used for the separation and quantitation of whey proteins. The separation was achieved using an alkyl C6 reverse phase column with an acidic saline/acetonitrile gradient. The major whey proteins were resolved completely in 30 minutes. In addition, genetic variants A and B of β -lactoglobulin were separated to better than 70% in the same analyses. Reproducibility of peak retention times and peak areas were 1 and 3%, respectively. Analyses of purified whey proteins revealed impurities not detected

by electrophoresis. Analysis was applicable to whey from a number of different sources of casein and cheese manufacture.

Whey protein isolates recovered by ion exchange and whey protein concentrates obtained by ultrafiltration were compared in terms of gross composition and in terms of more detailed protein content using both size exclusion and reverse phase HPLC (Barry et al., 1988). The size exclusion analysis was performed on a TSK G 300 column with eluting buffer of 0.05 M sodium phosphate, pH 7.4 containing 0.15 M sodium sulfate at a flow rate of 0.3 mL/min. Eluted proteins were detected by their absorbance at 280 nm. The HPLC separation was performed with a reverse phase Ultrasphere RPSC column containing 5 μ m particle size C3 propyl bonded phase. Solvent A was 0.15 M sodium chloride/HCl pH 2.1 and solvent B was acetonitrile. The gradient program was held at 0% B for 4 minutes, 0–30% B in 3 minutes, 30–42% B in 24 minutes, 42–0% B in 4 minutes.

Immunoturbidimetric methods have also been used to measure the whey protein content of milk and buttermilk powders (Greiner et al., 1985). Antibodies to whole bovine whey were developed for rapid screening of whey protein in nonfat dry milk and buttermilk. Milk samples are heat treated prior to analysis to denature the whey proteins for a more uniform response to antibodies. Of the whey proteins tested the assay is most sensitive to BSA and least sensitive to β -lactoglobulin. Precision of the method is about 4% coefficient of variation with a minimum level of detection of 3% whey protein concentrate added to nonfat dry milk.

Kim et al. (1987) determined the β -lactoglobulin, α -lactalbumin, and BSA contents of eight whey protein concentrate samples using reverse phase HPLC and by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The chromatographic column used was a C4 bonded reverse phase column with 300 Å pore size. A nonlinear gradient was used consisting of 30–45% acetonitrile containing 0.1% trifluoroacetic acid. The absorbance was measured at four different wavelengths of 210, 237, 250, and 280 nm. The coefficient of variation was 3.87% for BSA, 4.39% for α -lactalbumin, and 6.29% for β -lactoglobulin.

HPLC has also been used to determine the denaturation of whey proteins (Parris and Baginski, 1991). Denatured whey protein and casein were isolated from undenatured whey protein by isoelectric precipitation at pH 4.6. Whey protein denaturation was determined by comparing reverse phase

HPLC protein profile of isolates of heat-treated and unheated nonfat dry milk. In general, protein profile for heat-treated skim milk indicated whey protein denaturation began at 40°C, became more rapid at 70°C and was 95% complete, became more rapid at 70°C and was 95% complete at 85°C. Undenatured whey protein was also quantified as whey protein nitrogen based on their absorbance and nitrogen content compared to known whey protein standards or by augmenting the same samples with a known amount of lysozyme. Whey protein nitrogen values were obtained by a modified Kjeldahl nitrogen procedure. A C4 reverse phase bonded column of 10 mm particle size was used with solvent A being 0.1% trifluoroacetic acid in water and solvent B being acetonitrile. Absorbance at 280 nm was monitored to detect protein elution.

α -Lactalbumin, β -lactoglobulin, and BSA in raw and ultra-high temperature pasteurized milks were determined using capillary electrophoresis (Cifuentes et al., 1993). The separation buffer contained 40 mM Tris boric acid, 0.1% sodium dodecyl sulfate, and 10% polyethylene glycol 8000. Detection of the separated components was performed by monitoring the absorbance at 214 nm. The migration times were reproducible and results agreed well with high-performance liquid chromatographic separations.

Uncoated capillaries were used in the quantitation of whey proteins by capillary electrophoresis (Recio et al., 1995). Separations were performed using 100 mM borate buffer, pH 8.2 containing 30 mM sodium sulfate. The use of high pH and high ionic strength buffer reduced adsorption of proteins on the capillary walls making their separation possible. Reproducibility of migration times and peak areas are improved by optimizing the capillary equilibration procedure and by an internal standard. Relative standard deviations ranging between 0.74 and 1.03% for migration times and 2.14–5.23% for areas of major components are obtained. Detection limit of equal to or less than 0.5 mg/100 mL was achieved. Linear relationships of peak area to concentration have been used to quantitate BSA, α -lactalbumin, β -lactoglobulin A, and β -lactoglobulin B in cow's milk subjected to various thermal treatments.

Capillary zone electrophoresis has also been successfully applied to the quantitation of whey proteins in heat-treated milk (Recio and Olleman, 1996). The amount of denatured whey protein in heat-treated skim milk could be estimated by analyzing the casein fraction obtained by isoelectric precipitation at pH 4.6. A hydrophilic coated capillary was used in

combination with 6 M urea in citrate buffer at pH 3. Optimization of the sample and running buffer minimized the adsorption of serum proteins, especially that of BSA. This afforded a detection limit down to about 5–65 µg/mL for the three main components in milk serum. The detector response at 214 nm was linear in the range of 0.05–0.35 and 0.05–0.85 mg/mL for α -lactalbumin and β -lactoglobulin, respectively. BSA showed a slightly less linear behavior due to residual adsorption to capillary walls. The recovery of serum proteins was in the range of 89–107%.

A capillary electrophoresis method for the determination of casein and whey protein has also been reported (Miralles et al., 2001). The effects of several parameters such as pH, ionic strength, concentration of urea, and applied voltage on time and separation efficiency were studied. Using a hydrophilic coated capillary in combination with an electrophoresis buffer of 0.48 M citric acid–0.13 mM citrate containing 4.8 M urea at pH 2.3 and a separation voltage of 25 kV allowed for complete separation of β -lactoglobulin and para- κ -casein permitting the quantitation of both compounds.

What the preceding examples show are that there are a number of methods available for the detection and quantitation of whey proteins. Is there a preferred method that is recommended? A study was conducted to compare the three common analytical methods of polyacrylamide gel electrophoresis, HPLC, and capillary electrophoresis (Norris et al., 1998). The electrophoretic procedures included native polyacrylamide gel electrophoresis (native PAGE) and polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE). The capillary electrophoresis procedures used were capillary electrophoresis (CE), capillary gradient electrophoresis (CGE), capillary zone electrophoresis (CZE), and the HPLC procedures included size exclusion, ion exchange, reverse phase, and IgG affinity HPLC. The best method depends upon which component is critical in the analysis. For α -lactalbumin, the preferred methods in order of preference were reverse phase HPLC, size exclusion HPLC, reduced PAGE, and nonreduced PAGE. For β -lactoglobulin, reverse phase HPLC and reduced SDS-PAGE were found to be the most suitable. For BSA, size exclusion and native PAGE were deemed the best and for IgG, reduced SDS-PAGE and affinity protein G HPLC were optimal.

In another comparative study, CE, SDS-CE, and UV 4th derivative spectra were compared for their sensitivities and efficiency of quantitation of whey proteins (Miralles et al., 2000). Samples tested in

this study were raw milk and heat-treated milk. All methods effectively measured the whey protein to total protein ratios independently of the heat treatment applied to the samples. Mean values obtained by CE, SDS-CE and 4th derivative UV spectroscopy were respectively 17.1, 18.5, 17.3 for raw milk samples, 16.6, 17.7, and 18.8% for pasteurized milks, and 16.8, 17.0, and 17.2 for ultra-high temperature treated milks. The composition or states of the proteins in whey were not determined in this study.

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16

Ice Cream and Frozen Desserts*

Arun Kilara and Ramesh C. Chandan

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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 toward the end of the nineteenth century it was sold on the streets of metropolitan areas.

Ice cream and frozen desserts are popular throughout the world. Although ice cream is a popular frozen dessert in all parts of the world, the major consumer countries of ice cream are New Zealand, the United States, Canada, Australia, Belgium, Finland, and Sweden. In some areas of the world, unavailability of appropriate ingredients, lack of refrigerated distribution chain, economics or other cultural factors may deter the manufacture (and therefore consumption) of ice cream. There are several different names for ice cream in the world. In Norwegian, it is *iskrem*, in Portuguese *sorvettes*, in Spanish *haldos*, in French *glace*, in Italian *gelato*, in Hebrew *glidah*, in German *eis*, in Finnish *jatelo*, in Greek *pagoto*, and in Chinese *bing qi lin* or *sou go*.

The popularity of ice cream results from several characteristics such as partial freezing, cooling, and refreshing sensation produced when the product is consumed, its sweet taste, and the lack of a preconditioning aroma. In all these products a sugar solution is the common denominator. The characteristics of the syrup are manipulated by the addition of other materials to obtain desired taste, texture, consistency, and appearance.

* Revised and updated the chapter by Kilara and Chandan, 2007.

Ice cream is manufactured as regular, custard/French, reduced fat, light, low and no fat versions. Other frozen desserts include frozen yogurt, sherbet, water ice, 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. Similarly, the degree of freezing results in products that are hard frozen, or designed for dipping or scooping, or used as soft serve or a milk shake. The characterizing materials that are added to the sugar syrup are dairy ingredients, sweeteners (other than sucrose), body and texture modifiers, flavors, and colors. Most of the legal terms used in this chapter are based on the authors' experience in the United States and it is recognized that this information varies considerably due to geography.

TRADE CLASSIFICATION OF ICE CREAM

The chemical composition of ice cream differs mainly with regard to the fat content and three grades of ice cream can usually be found in most market areas. One grade just meets the minimum fat content, often has an overrun 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, 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.

TRENDS IN ICE CREAM MARKET

The volumes of ice cream and other frozen dairy desserts produced in the United States in 2005 are shown in Table 16.1.

According to United States Department of Agriculture statistics, 1.535 billion gallons of ice cream and frozen desserts were manufactured in 2005. Of

Table 16.1. Production Statistics of Various Frozen Desserts in the United States

Frozen Dessert	2005 (Million Gallons)
Ice cream, regular, total	888.7
Ice cream, low-/nonfat, total	109.9
Sherbet	55.3
Water juices and ices	66.1
Frozen yogurt, total	29.6
Other frozen dairy desserts	7.7

Source: <http://www.usda.gov/mass/pubs/agro5/05-ch8.PDF>.

this total, 62% was ice cream, 25% was reduced fat, low-fat, and nonfat ice cream, 1.9% frozen yogurt, 4.3% water and juices, 3.8% sherbet, and 0.5% other frozen products. It is estimated that 358.7 million gallons of soft serve ice cream products were produced in 2005. In the United States, per capita sales in 2004 were 26.2 lb. and the market value exceeded 21.6 billion dollars.

FORMULATION

In order 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 (aka serum solids), and the third is called a balancing ingredient.

Representative formulae for commercial grades of ice cream are shown in Table 16.2.

Composition of other variants of ice cream and frozen desserts are shown in Table 16.3.

The prioritization of ingredient selection can be said to approximate the hierarchy as follows:

1. *Select milk fat content*: Since milk is bought and sold on the basis of fat content and quality grade of ice cream is also reflected by the fat content, the amount of milk fat will determine the cost and grade of the product.
2. *Select nonfat milk solids level to complement the fat content*: Too much of nonfat milk solids can cause sandiness.
3. *Sweetener ingredient*: Type and amount of sweetener are in turn dependent on the fat and total solids content and also by economic considerations. Higher fat mixes need high sweetener levels. Type of sweetener used depends on demands and supply which in turn controls the cost of the ingredients.
4. *Stabilizer and emulsifier*: The amount and type are affected by fat and total solids levels,

Table 16.2. Representative Formulae for Ice Creams of Different Grades

Constituent	Grades of Ice Cream (%)				
	Minimum Standard	Regular	Premium		Super Premium
			1	2	
Milk fat	10.00	12.00	14.00	16.00	18.00
Milk solids non fat	7.50	9.00	10.00	10.50	9.50
Whey solids	2.50	2.00	—	—	—
Sucrose	4.50	7.60	12.00	15.00	15.00
Corn syrup solids	9.00	6.80	5.00	—	—
High fructose solids	4.50	2.60	—	—	—
Stabilizer	0.35	0.25	0.13	0.12	—
Emulsifier	0.25	0.25	0.15	0.10	—
Total solids	38.70	40.50	41.28	41.72	42.50

manufacturing processes, and storage and distribution factors.

5. *Label considerations:* If specific nutritional or other claims have to be made (e.g., “All Natural” or “Reduced Calorie” or “Reduced fat”) then the above hierarchy of ingredient selection is slightly modified and cost of ingredients plays a lesser role than the functional aspects.

For the manufacture of good ice cream there is no secret or magic formula. Difference between good and not-so-good ice cream is controlled management. By controlled management is meant such things as using good ingredients for every batch, maintaining the same composition every time, proper and controlled mix making, freezing, packaging, hardening, storage, distribution, and minimal turnover time.

These representative subclasses vary not only in the proportion of ingredients but also in the types of ingredients used to furnish a required component. For example, in the “Economy” subclass of ice cream serum solids may be derived from nonfat dry milk (skim milk powder), or condensed skim milk and can also include the maximum allowable amounts of whey solids. The maximum allowed amount of whey solids is 25% of the milk solids-not-fat (MSNF) content. Therefore, the 10.0 % of serum solids may be derived from condensed skim or nonfat dry milk up to a total of 7.5% and whey solids may be added to the level of 2.5%. Because whey solids cost considerably less than milk solids, thereby the overall costs of the formulation can be reduced. Similarly, sweeteners may also be substituted to reduce costs of formulations. High fructose corn syrup has approximately

Table 16.3. Composition of Other Frozen Desserts

Constituent	Ice Cream				
	Nonfat	Low-Fat	Reduced Fat	Sherbet	Water Ice
	Abundance (%)				
Milk fat	0.5	3.0	6.0	1.5	0
MSNF	13.5	13.0	12.5	3.5	0
Sucrose	10.0	9.0	10.0	23.0	23.0
Corn syrup solids	10.0	9.0	8.0	7.0	7.0
Stabilizer/emulsifier	1.0	0.8	0.6	0.4	0.4
Total solids	35.0	34.8	37.1	35.4	30.4
Water	65.0	65.2	62.9	64.6	69.6

the same sweetness intensity as sugar but costs less than sugar. In some formulations, approximately 50% of the sugar solids can be substituted with high fructose corn syrup solids. In general, the “Economy,” “Regular,” and “Premium” subclasses of ice cream also contain higher overrun while the “Super Premium” subclass contains less overrun. Additionally, the “Super Premium” subclasses are packaged in smaller containers of 468 mL (1-pint) whereas the other products are packaged in 1.875 liters (U.S. 0.5 gallon) containers. Lastly, the distribution and points of sales of the different subclasses of products can also vary considerably.

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 membrane called milk fat globule membrane. This fat is made up of triglycerides, traces of di- and monoglycerides, cholesterol, and phospholipids among many other substances. In fact, milk fat is composed of some 3,600 different compounds. The triglycerides which are the main component are synthesized by the cow by linking three molecules of fatty acids to one molecule of glycerol, hence the name triglycerides. These fatty acids consist of carbon, hydrogen, and oxygen atoms. Fatty acids can have as few as 4 carbon atoms or as many as 26 carbon atoms. In milk fat, we have a number of different fatty acids including those containing 4, 6, and 8 carbon atoms. This is significant because the characteristic fl vor of milk fat is in large part due to the presence of these lower chain fatty acids butyric, caproic, caprylic, and capric acids, carbon numbers 4, 6, 8, and 10, respectively. Further there are fatty acids that are unsaturated and thus we have fatty acids with one double bond (monoenoic) fatty acids, two double bonds (dienoic), or with three fatty acids (trienoic). The unsaturated fatty acids with multiple double bonds are functional, healthy, and essential for human beings.

Milk fat melts and crystallizes, is unctuous, depresses the cold sensation, contributes desirable flavor, is a solvent for added fl vors, adds structure to ice cream and is of great importance in extrusion properties of ice cream. Extrusion helps shape ice cream in novelties.

Milk and cream constitute the most important components because they furnish the basic ingredients for a good quality ice cream. Variables related to dairy ingredients exert a profound influence on the fl vor, body, and texture of the frozen product.

The nature and intensity of ice cream fl vor is a function of the fl vor 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. Actually, fl vor problems could 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.

Balancing quality and cost is a major challenge to frozen dessert manufacturer. Satisfactory composition produces ice cream having an optimum combination of cost, fl vor, body, and texture, cooling effect, viscosity, whipping ability, and freezing characteristics. In summary, factors responsible for overall ice cream quality are raw material quality, sanitary care during mix preparation, processing parameters, fl voring used, freezing techniques, and storage conditions.

Formulation of frozen dessert mix involves utilization of both the fat and solids-not-fat components of milk. The functions and preferred sources of major ice cream ingredients are summarized in Table 16.4.

Sweet fresh cream and fresh milk: Whole milk may be used primarily as a source of milk solids. Without question, there is no better source of fat than sweet cream because of its desirable fl vor, convenience of handling, and good whipping characteristics. Fresh cream is judged by fl vor, 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.

Frozen cream: 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 insure prevention of the development of off-fl vors in stored cream. Only the best cream should be processed for storage, and it should contain no developed acidity. Off-fl vors 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 butter fat brought about by enzymatic activity of lipase on the butterfat or by

Table 16.4. Role and Sources of Various Components of Ice Cream

	Role and Functions	Limitation	Sources In Order of Preference
Milk fat	Imparts desirable creamy rich fl vor Source of fat-soluble vitamins Improves body texture Improves melting resistance	Calorie dense. Too much fat (17%) gives too much viscosity to mix and hinders whipability Source of oxidized, rancid, and fish 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 fl vor	Fluid whole milk Fluid skim milk Condensed skim milk Skim milk powder Whey products
Sweeteners	Impart sweet fl vor	Too much sweetener, especially corn syrup impedes freezing process	Cane sugar, corn syrup solids, high fructose corn syrup

certain bacteria of the enzymes they produce. A proper heat treatment regime, an essential phase for the preparation of cream for freezing, consists of heating cream at 76.7°C for 20 minutes, or 82.2°C for 10 minutes, or 87.8°C for 5 minutes. This treatment not only inactivates the lipase enzyme naturally present in milk but also destroys 95–99% of the bacteria present.

Heat treatment of cream also increases the resistance of the cream to oxidation. A fis y fl vor in dairy products results from the formation of trimethylamines by the hydrolysis and oxidation of lecithin, naturally occurring phospholipid in milk. Factors that promote development of this fl vor are high acidity and the presence of prooxidants (iron or copper salts). Evidently, heat treatment at these 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. Apparently, these sulfhydryl groups function as antioxidants in the liquid system. In addition, they may complex with prooxidant minerals.

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.

Because of fluctuating supplies and 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 oil-in-water emulsion. The aqueous phase is skim milk. Agitation of cream results in a phase inversion converting oil-in-water emulsion to water-in-oil emulsion. The serum is separated and is called buttermilk. This buttermilk is not to be confused with cultured buttermilk which is a different product. Buttermilk is dried into a powder form 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, it requires melting and is considered a processing inconvenience.

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 removing moisture and residual serum solids from unsalted butter. Butteroil is sold in 55 gallon drums and is stored under ambient conditions. During storage, however, 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.

All other things being equal, the preferred sources of concentrated milk fat (from most to least) are cream, unsalted butter, and butteroil. Choice depends

upon availability, economics, local preferences, regulatory factors, and quality of the ingredients.

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). Surfactancy of proteins results in desirable interfacial behavior.

Fluid whole and skim milk: Both are excellent sources and should be used in the mix. However, because of their low serum solids content in contrast to the serum solids desired in ice cream mix, their use is limited. Skim milk should be purchased on the basis of a definite MSNF content in order to guard against dilution with water.

Plain condensed skim milk: Fresh condensed skim milk is easy and convenient to use, has an excellent flavor. The concentrate may be paid for on the basis of the solids content, which runs 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: This is concentrated about two and a half times and contains 8% fat and 20% serum solids.

Superheated condensed skim or whole milk: The use of superheated condensed milk may substitute the use of heat concentrated milk. The already-condensed product is slowly heated to a high temperature, usually in the range of 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.

Sweetened condensed whole or skim milk: A sweetened condensed product 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%).

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.

SWEETENERS

Ice cream is a sweet frozen dessert. Its sweetness is due to the presence of sugars and other sweeteners. Sweeteners are classified either as nutritive or non-nutritive.

Nutritive Sweeteners

They 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), and high fructose corn syrups, sugar alcohols (xylitol, maltitol, sorbitol, and glycerol). A comparison of the properties of nutritive sweeteners is given in Table 16.5.

Sugar (sucrose): It provides sweetness, depresses 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: Liquid sugar is sugar syrup containing 67% sugar and 33% water. It is used by high

Table 16.5. Comparison of Properties of Nutritive Sweeteners

Sweetener	Relative Sweetness	Solubility (Grams/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 & 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	Mixture
Corn syrup solids (42 DE)	0.45	70	Mixture
High fructose corn syrup	1.2	67	Mixture

volume ice cream manufacturers and is sold in rail tank cars or truck load quantities. It can be easily pumped and metered into ice cream mix making operations.

Monosaccharides: 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, 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: Sorbitol and xylitol are examples of sugar alcohols. They depress freezing point to an extent greater than disaccharides and similar to monosaccharides. Glycerol depresses the freezing point to a greater extent than sugar alcohols and ethanol (alcohol) depresses the freezing point to an extent greater than glycerol. These are illustrated in Table 16.6.

Corn sweeteners: 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 equivalents (DEs). DE is a measure of the extent of hydrolysis or modification of starch. Regular conversion syrups are 38–38 DE, intermediate conversion syrups are 48–58 DE, and high conversions 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.

Other nutritive sweeteners: Honey is used as a sweetener. Honey is like invert sugars. It is made up of glucose and fructose, both monosaccharides, therefore it depresses freezing point to a greater extent than sugar at equivalent concentrations.

Maltodextrins: In certain low-fat and no-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 freezing point of the mix. These products are also derived from corn starch and the modification of the starch is to a much lesser extent than with maltodextrins. Typically the DE ranges from 5 to 15 for maltodextrins.

Nonnutritive Sweeteners

No sugar added products are ice cream and frozen desserts in which no sugars are added to achieve sweetness. Such formulations rely on the addition of intense nonnutritive sweeteners. The intense sweeteners that do not provide any significant calories at use levels. They include sucralose, aspartame,

Table 16.6. 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

Table 16.7. Comparisons Among Nonnutritive Sweeteners

Sweetener at 25°C	Relative Sweetness	Solubility (Grams/100 g)
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

saccharin, cyclamates, Acesulfame-K, and many others. As little as 0.07% aspartame can provide the sweetness equivalent to 15% sugar. By reducing the mass of the formulation by 14.93% we have more water in the formulation to control. In order 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 levels of stabilizers in the formulation. A comparison of the nonnutritive sweeteners is provided in Table 16.7.

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 27°F (2.7°C).

STABILIZERS AND EMULSIFIERS

Apart from sweeteners, stabilizers and emulsifier are also important nondairy ingredients.

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. By 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, 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, it controls 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 level 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 method, more stabilizer may be needed than if the same mix were pasteurized by the batch method or by ultra-high temperature method.

A good stabilizer should be nontoxic, 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. Some of the common stabilizers and their characteristics are listed below:

Gelatin: It is an animal protein derivative and is effective at high concentrations of 0.3–0.5% and is expensive and therefore rarely used in the United States. 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.

Guar gum: This stabilizer 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 carboxymethyl cellulose (CMC): This is a chemical derivative of cellulose. Cellulose is the most abundant carbohydrate in nature. CMC 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: This 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: This stabilizer 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.

Emulsifier

As opposed to stabilizers, emulsifiers exert their action on the fat phase of ice cream. Emulsifiers are surface active agents (surfactant). Fat and water do not mix. 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, mono- and diglycerides and ethoxylated esters of sorbitol (polysorbates) are the commonly used emulsifiers. Mono- and diglycerides are derived from fatty acids and glycerol. Therefore, emulsifiers are fatty substances. They also show fat-like properties of melting point, crystallinity, and they can be composed of saturated or unsaturated fatty acids. Presence of emulsifier in ice cream leads to smoother texture and better shape retention, while improving the ability of the mix to incorporate air.

Mono- and diglyceride mixtures: These compounds are obtained by the chemical treatment of fats such as lard, palm kernel, or soybean oil. Most of the mono- and diglycerides are solid at room temperature and are added to the mix prior to pasteurization at a level of 0.1–0.2%. Emulsifier with high monoglyceride content are also effective drying agents.

Polysorbates: Polysorbates are polyoxyethylene compounds. These synthetic chemicals are the most effective drying agents. Polysorbate 80 is an oleic acid derivative. It is very powerful drying agent and is used at 0.04–0.07% levels. Polysorbate 65 is helpful as a whipping agent, that is, 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.

Egg products: 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: Buttermilk powder provides phospholipids which can act as emulsifiers

Mix Calculations

Once a formula has been finalized a recipe has to be created. Formulas specify composition of the desired mix in terms of percentages of fat, MSNF, sweeteners, stabilizers, and emulsifiers. A recipe calculates the weight and/or volumes of ingredients needed to meet the formula requirements. These calculations are called mix calculations.

Mix calculations are essential for manufacturing consistent quality finished products. When composition of raw materials varies or the economics of ingredients changes, the recipe for making an ice cream mix has to change. Further this change has to occur in a manner that the finished product composition is not altered. In some instances changing regulatory definition and health claims may necessitate manufacture of products to carefully define specifications. Mix calculations are important in standardizing mixes prior to freezing. Ice cream plants now use computer software programs to calculate the amounts of various ingredients to conform to required specification of composition of ice cream mix. However, for basic understanding, we will discuss below the fundamentals of the mix calculations.

Mix calculations can be performed by Pearson's Square, algebraic methods, and arithmetic methods. Pearson's Square is of limited utility, algebraic methods are complicated and involve the use of simultaneous equations and matrices. Arithmetic calculations are simpler and require fewer computations than algebraic method. In this section, all three methods will be discussed. Prior to calculating a mix, certain preliminary steps have to be completed.

Several factors are important in mix calculations:

1. Composition of the mix has to be specified. Mix composition clearly states the percentage of fat, MSNF, sugar, other sweeteners, stabilizers and emulsifiers and alternate ingredients. For example, a typical mix composition would be milk fat 10.1%, MSNF 10%, sugar 8.0%, high fructose corn syrup 4%, corn syrup solids (36-DE) 5.0%, stabilizer 0.3%, and emulsifier 0.2%. The total solids of this mix are 37.6%. Although not specified explicitly, the water content of this mix is $100 - 37.6 = 62.4\%$.
2. The source of the ingredients should also be specified. For example, milk fat will be derived from cream, MSNF from condensed skim milk, and so forth.
3. The amount of mix to be made must also be determined. Generally, if volumetric amounts are known (e.g., 100 liters or 100 gallons), these quantities have to be converted to weight equivalents of kilograms or pounds. Specific gravity or density of the mix would have to be known.
4. In order to make an ice cream mix, three categories of ingredients are essential. These are (a) a concentrated source of milk fat, (b) a concentrated source of milk MSNF, and (c) a balancing ingredient.
5. In order to perform mix calculations, determine the composition of the mix to be made, list the available ingredients and their compositions, and list the amount of mix to be made in weight equivalents. Then perform calculations.
6. After performing calculations, verify or validate the calculations by constructing a proof sheet.
7. Make the mix according to calculated amounts of the ingredients and test to make sure that the desired composition has been attained. If required, standardize the mix to obtain the desired composition.

Terms used in mix calculations: Serum in dairy ingredients (milk, skim milk, cream, condensed skim milk, condensed whole, etc.) is the fat-free portion of that ingredient.

$$\% \text{ Serum} = 100 - \% \text{ milk fat}$$

$$\% \text{ Serum} = \% \text{ milk solids-not-fat} + \% \text{ moisture}$$

If fat is considered to be 0 in skim milk, serum in skim milk is $100 - 0 = 100$

Serum in 3.5% fat milk is $100 - 3.5 = 96.5$

Serum in nonfat dry milk (skim milk powder, assuming 0% fat) is $100 - 0 = 100$

Serum in sweetened condensed skim milk with 0% fat, 25% sugar is $100 - 25 = 75$

Serum in sweetened condensed whole milk with 8% fat and 40% sugar is $100 - (8 + 40) = 52$

Serum in ice cream mix is calculated slightly differently than serum in milk ingredients.

$$\% \text{ Serum in ice cream mix} = 100 - (\% \text{ milk fat} + \% \text{ sweeteners} + \% \text{ stabilizers/emulsifier} + \text{other nondairy solids})$$

Serum in an ice cream mix with 10.1% fat, 10% MSNF, 8% sugar, 4% high fructose corn syrup, 5% corn syrup solids, and 0.3% stabilizer and 0.2% emulsifier is $100 - (10.1 + 8 + 4 + 5 + 0.3 + 0.2) = 100 - 27.6 = 72.4$.

$$\text{Weight of product} \times \% \text{ of constituent as a decimal} = \text{weight of a constituent.}$$

Example 1: Make 100 lb. mix to contain 15% sugar, 0.35% stabilizer, 0.5% egg yolk solids, 12.5% fat, and 11% serum solids. Available ingredients are 40% fat cream, 3.5% fat milk, 8% fat, and 20% serum solids condensed milk.

Solution: For 100 lb. mix, 15 lb. sugar, 0.35 lb. stabilizer, and 0.5 lb. egg yolk solids are needed. Therefore, $100 - (15 + 0.35 + 0.5) = 84.15$ lb. has to be derived from cream and condensed milk.

Let $x = 40\%$ cream, $y = 3.5\%$ milk, and $z =$ condensed milk. It follows that

$$x + y + z = 84.15. \quad (16.1)$$

We know that the total fat content must be 12.5 lb. Therefore,

$$0.4x + 0.035y + 0.08z = 12.5. \quad (16.2)$$

Also, we know that serum solids needed is 11%, so

$$0.053x + 0.085y + 0.20z = 11. \quad (16.3)$$

Thus, we have three simultaneous equations for three unknowns. Eliminate an unknown by expressing in terms of other unknowns.

$$x + y + z = 84.15$$

$$x = 84.15 - y - z. \quad (16.1a)$$

Substituting this value for x in (16.2) we get

$$0.40(84.15 - y - z) + 0.035y + 0.08z = 12.5. \quad (16.2a)$$

Simplifying (16.2a), it converts to

$$36.5y + 32z = 2116. \quad (16.2b)$$

Similarly, (16.3a) becomes

$$0.053(84.15 - y - z) + 0.085y + 0.20z = 11. \quad (16.3a)$$

Conversion of (16.3a) results in

$$3.2y + 14.7z = 654.005. \quad (16.3b)$$

If we wish to eliminate one of the equations in (16.2b) and (16.3b) it is necessary that the multiples of unknowns which we wish to eliminate be the same in both equations. If we wish to eliminate y , then the multiples of y must be the same in both equations. This condition is obtained as follows:

Multiply (16.2b) by 3.2 to obtain (16.2c) and multiply (16.3b) by 36.5 to obtain (16.3c).

$$18y + 102.4z = 6771.2 \quad (16.2c)$$

$$18y + 536.55z = 23871.18 \quad (16.3c)$$

Subtract (16.3c) from (16.2c) and the result is

$$434.15z = 17099.98 \quad (16.4)$$

$$z = 17099.98/434.15 = 39.39 \text{ lb. of condensed milk.}$$

Solve for y by substituting this value for z in either (16.2b) or (16.3b). Substituting in (16.2b) we get:

$$36.5y + (32 \times 39.39) = 2,116$$

$$36.5y = 855.52$$

$$y = 855.52/36.5 = 23.44 \text{ lb. 3.5\% fat milk.}$$

By substituting found values of y and z in (16.1) we get:

$$x + 23.44 + 39.39 = 84.15$$

$$x + 84.15 - (23.44 + 39.39) = 84.15 - 62.83 \\ = 21.32 \text{ lb. cream.}$$

To validate the calculation let us construct a proof table.

Weight	Ingredient	Fat	Serum Solids
15.0	Sugar	0.00	0.00
0.035	Stabilizer	0.00	0.00
0.50	Egg yolk solids	0.00	0.00
21.32	Cream	8.5280	1.1299
23.44	Milk	0.8204	1.9924
39.39	Condensed milk	3.1512	7.8780
100.00	<i>Total calculated</i>	12.4996	11.0003
100.00	<i>Total desired</i>	2.5000	11.0000

Arithmetic method: This method relies on two main types of equations. One is called the serum point formula and is useful when fat is derived from only one ingredient. The other is called milk and cream formula and is used when multiple sources of fat are involved.

1. *Serum point formula:* This equation is used to calculate the amount of concentrated source of serum solids need for a mix recipe.

$$\frac{\text{lbs. serum solids needed} - (\text{lbs. serum of mix}) \times 0.09}{\text{lbs. serum solids of 1 lb. condensed} - (\text{lbs. serum of 1 lb. condensed}) \times 0.09}$$

The terms serum solids needed are given in mix composition, the word condensed simply means concentrated source of milk solids-not-fat. This equation contains both terms *serum* and *serum solids*.

2. *Milk and cream formula:*

$$\frac{\text{lb. fat needed} - (\text{lb. milk} + \text{cream needed}) \times (\text{lb. fat of 1 lb. milk})}{\text{lb. fat of 1 lb cream} - \text{lb. fat of 1 lb. milk}}$$

The milk and cream formula is used in instances where fat is supplied by more than one source in the same recipe.

This is an exercise for fillin out a Mix Proof Sheet, a 1,000 lb. ice cream mix is composed of the following ingredients:

Ingredient	Pounds
40% fat cream	237.3
4% fat milk	376.9
29% S/MSNF condensed skim milk	222.8
Sugar	160.0
Stabilizer/emulsifie	3.0

- (a) Enter the pounds of each ingredient into a proof table.

- (b) Calculate the pounds of each constituent contributed by each ingredient. Constituents are fat, MSNF, sugar, stabilize/emulsifie .
- (c) Total the pounds in each of the constituent columns of the proof sheet and calculate the percentage composition to the nearest hundredth percent of each of the mix constituents.

ANSWERS

- a. Pounds of milk fat from cream = $237.3 \times 0.40 = 94.92$ lb.
- b. % Serum solids in cream = $100 - 40 = 60 \times 0.09 = 5.4\%$
- c. Pounds of serum solids in cream = $237.3 \times 0.054 = 12.81$ lb.
- d. Pounds of total solids in cream = $94.92 + 12.81 = 107.73$ lb.
- e. Pounds of fat from milk = $376.9 \times 0.04 = 15.08$ lb.
- f. % Serum solids in milk = $100 - 4 = 96 \times 0.09 = 8.64\%$
- g. Pounds of serum solids in milk = $376.9 \times 0.0864 = 32.56$ lb.
- h. Pounds of total solids in milk = $15.08 + 32.56 = 47.64$ lb.
- I. Pounds of milk fat in condensed skim milk = 0.0
- j. Pounds of serum solids in condensed skim milk = $222.8 \times 0.29 = 64.61$ lb.
- k. Pounds of total solids in condensed skim milk = 64.61 lb.
- l. Pounds of sugar = 160 lb.
- m. Pounds total solids of sugar = 160 lb.

- n. Pounds of stabilizer/emulsifie = 3 lb.
- o. Pounds of total solids of stabilizer/emulsifie = 3 lb.
- p. Total Pounds of mix = $237.3 + 376.9 + 222.8 + 160 + 3.0 = 1000$
- q. Total Pounds of Milk Fat = $94.92 + 15.08 = 110.0$
- r. % Milk fat = $110/1,000(100) = 11.0\%$
- s. Total pounds of serum solids = $12.81 + 32.56 + 64.61 = 109.98$
- t. % Serum solids = $109.98/1,000(100) = 10.998$ or 11.0%
- u. Total pounds of sugar = 160
- v. % Sugar = $160/1,000(100) = 16\%$
- w. Total pounds of stabilizer/emulsifie = 3
- x. % Stabilizer/emulsifie = $3/1,000(100) = 0.3\%$
- y. Total Pounds of solids in mix = 382.98
- z. % Total solids of mix = $382.98/1,000(100) = 38.298$ or 38.3%

Mix POOF SHEET

Desired Formula	Available Ingredients
—% Milk Fat 237.3 lb.	40% fat cream
—% Milk solids-not-fat 376.9 lb.	4% fat milk
—% Sucrose 222.8 lb.	Condensed milk 29% MSNF (SS)
—% Corn syrup solids 160.0 lb.	Sugar
—% Stabilizer 3.0 lb.	Stabilizer/emulsifie
—% Emulsifie	
—% Total solids	
—lb. of mix	

Ingredient	Quantity (lb.)	Milk Fat (lb.)	Serum Solids (lb.)	Sucrose (lb.)	Corn Syrup Solids (lb.)	Stabilizer (lb.)	Emulsifie (lb.)	Total Solids (lb.)
40% fat cream	237.3	94.92	12.81					107.73
4% fat milk	376.9	15.08	32.56					47.64
29% MSNF condensed milk	222.8	0.0	64.61					64.61
Sugar	160.0			160.0				160.0
Stabilizer/emulsifie	3.0					3.0		3.0
Total	1000.0	110.0	109.98	160.0		3.0		382.98
Percent	100	11.0	11.0	16.0		0.3		38.3

Example 2: Calculate the amount of ingredients needed to make 100 lb. of an ice cream mix:

Desired Composition	Available Ingredients
10.00% Milk fat	30% fat cream
12.00% MSNF	skim milk
15.00% Sugar	Condensed Skim milk (27% MSNF)
0.30% Stabilizer/emulsifie	Sugar
37.3% Total Solids	Stabilizer/emulsifie

Fill in the attached proof sheet and provide all calculations supporting your solution.

- a. Pounds of serum solids needed = $100 \times 0.12 = 12.0$

Ingredient	Quantity (lb.)	Milk Fat (lb.)	Serum Solids (lb.)	Sucrose (lb.)	Corn Syrup Solids (lb.)	Stabilizer (lb.)	Emulsifie (lb.)	Total Solids (lb.)
30% fat cream	33.33	10.0	2.10					12.10
Skim milk	22.05		1.98					1.98
27% MSNF condensed skim	29.32		7.92					7.92
Sugar	15.0			15.0				15.00
Stabilizer/emulsifie	0.3					0.3		0.30
<i>Total</i>	100.0	10.00	12.00	15.00		0.30		37.30
<i>Percent</i>	100	10	12	15		0.3		37.30

- I. Pounds of skim milk required (by difference) = $100 - (33.33 + 29.32 + 15.0 + 0.3) = 22.05$
 j. Pounds serum solids in skim milk = $22.05 \times 0.09 = 1.9845$ or 1.98
 k. Pounds of sugar needed = $100 \times 0.15 = 15$
 l. Pounds of Stabilizer/emulsifie needed = $100 \times 0.003 = 0.3$

Desired Formula	Available Ingredients
10.0% Milk fat	30% fat cream
12.0% Milk solids-not-fat	Skim milk
15.0% Sucrose	27% MSNF condensed skim
0.0% Corn syrup solids	Sugar
0.3% Stabilizer/emulsifie	Stabilizer/emulsifie
% Emulsifie	
37.3% Total solids	
100 lb. of mix	

- b. Pounds of serum of mix = $100 - (10 + 15 + 0.3) = 100 - 25.3 = 74.7$
 c. Pounds of serum solids of 1 lb. condensed = $27/100 = 0.27$
 d. Pounds of condensed skim needed =

$$\frac{\text{lbs. serum solids needed} - (\text{lbs. serum of mix}) \times 0.09}{\text{lbs. serum solids of 1 lb. condensed} - (\text{lbs. serum of 1 lb condensed}) \times 0.09}$$

$$\{12 - (74.97 \times 0.09)/0.27 - (1.0 \times 0.09)\} = (12 - 6.723)/(0.27 - 0.09) = 29.32$$

- e. Pounds of cream needed = $10/0.3 = 33.33$
 f. % serum in 30% cream = $100 - 30 = 70$
 g. % Serum solids in cream = $70 \times 0.09 = 6.3$
 h. Pounds serum solids in cream = $33.33 \times 0.063 = 2.09979$ or 2.10

PROCESSING

Figure 16.1 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. Mix processing begins with the assembly of the necessary ingredients in the desired amounts. Generally, this assembly requires weighing the ingredients or if liquid

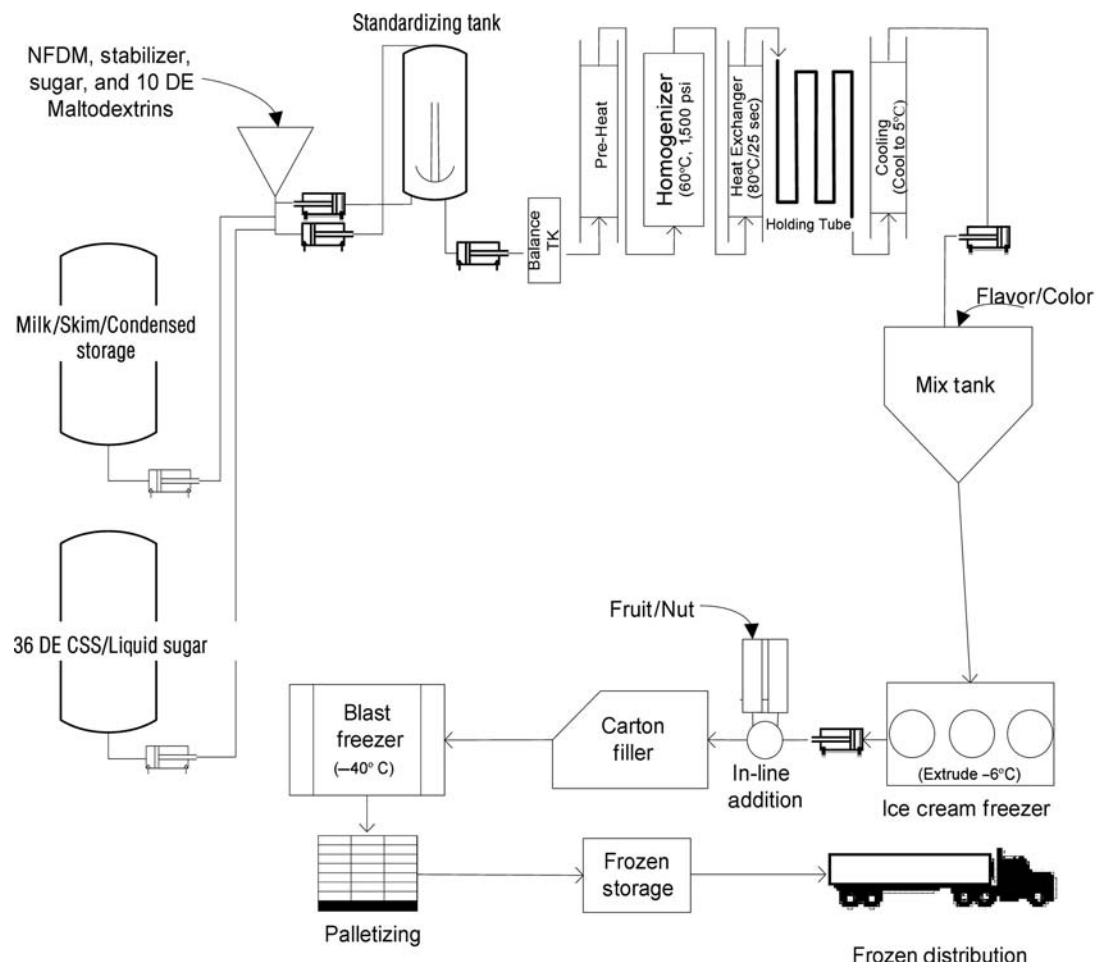


Figure 16.1. Flow sheet diagram for the manufacture of ice cream.

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.

BLENDING

In the next step the ingredients are blended together. Mix blending can be performed at refrigeration temperatures 40°F (4.4°C) or at warmer temperatures 113°F (45°C). Cold batching is a preferred method when cream, liquid milk, and condensed skim are the ingredients. Warmer temperatures are gener-

ally used when the ingredients include butter, but-teroil/anhydrous milk fat in combination with nonfat dry milk. Batching begins by placing a 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 emulsifier are incorporated into this liquid and finally cream is added. When cream is subjected to excessive shear it can be churned to butter. This should be avoided. Incorporation of the dry ingredients is aided by one of two types of devices viz. (a) powder horn and (b) high shear mixers. The powder horn is the easiest device and consists of a funnel with a valve placed in line with a

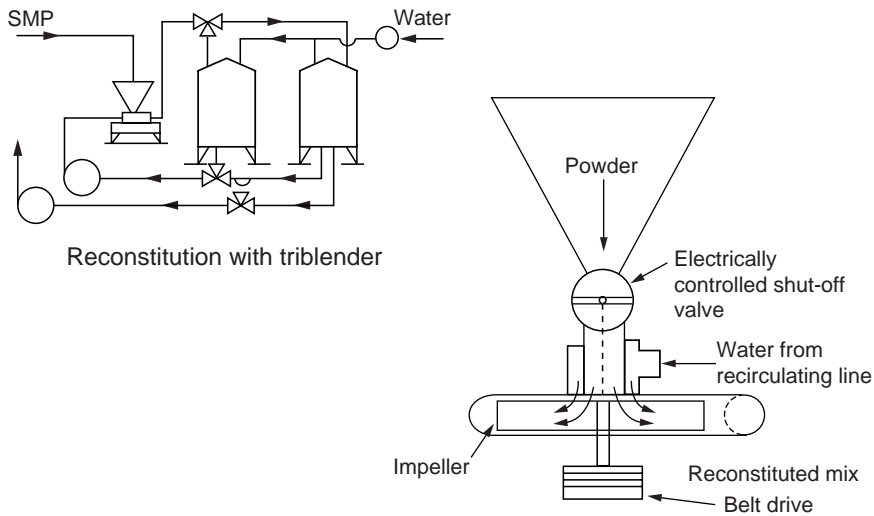


Figure 16.2. Incorporation of dry ingredients by a triblender system.

pump and capable of recirculating the liquid in the vat (Fig. 16.2).

With the valve closed, the dry ingredient is placed in the funnel, the pump is started and once the fluid is recirculated, the valve under the funnel is opened. As the liquid flows past the funnel it inspires (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 process fluid and then the valve beneath the funnel is closed. If this valve is not closed a large amount of air can be inspired into the mix creating foam which is undesirable.

In the second process (Fig. 16.3), a high shear mixer that functions like a giant Waring blender is used.

Here the process fluid is filled to 3/4th 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.

PASTEURIZATION

The hydrated ingredients are pasteurized and homogenized. Pasteurization is a heat treatment given to food products to destroy pathogenic (disease causing) microorganisms. According to the U.S. Public Health Service and its Pasteurized Milk Ordinance

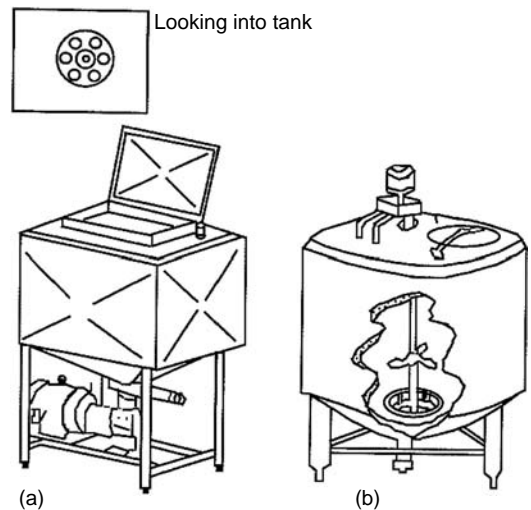


Figure 16.3. High shear mixing devices (a) Likwifier and (b) turbine blender.

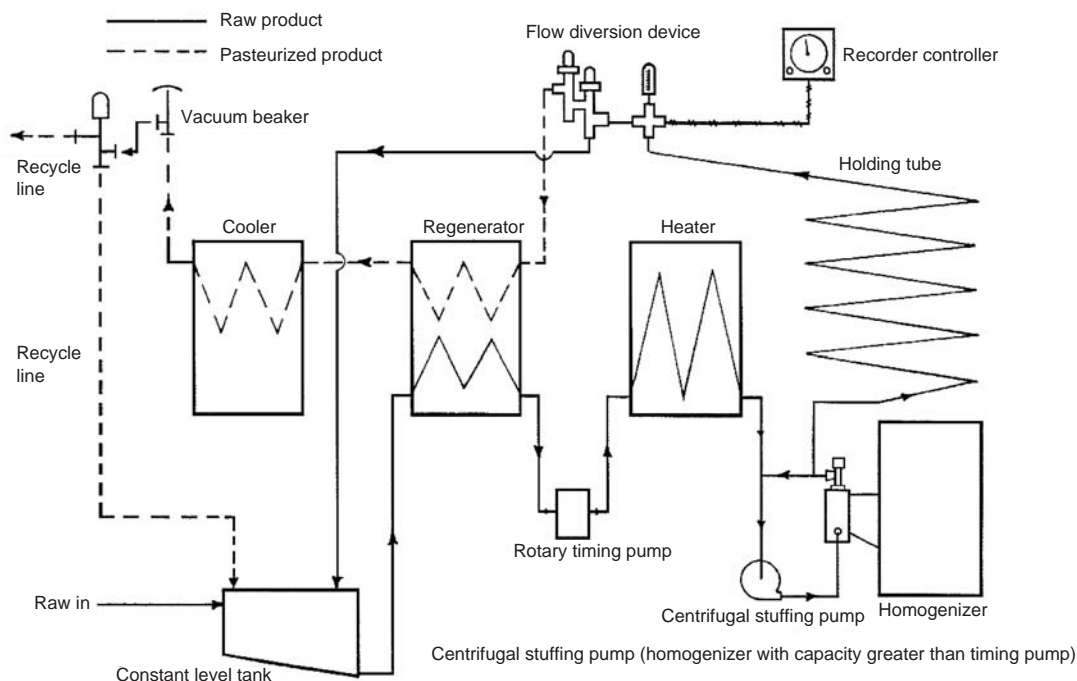


Figure 16.4. Schematic of a high-temperature short-time pasteurizer with timing pump and homogenizer.

pasteurization of an ice cream mix requires that every drop of mix be heated to 155°F (68.3°C) and held at that temperature for 30 minutes. Alternately, every drop of mix should be heated to 175°F (79.4°C) and held at that temperature for 25 seconds. Pasteurization can be performed either as a batch operation or as a continuous operation.

The 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) and once that temperature is attained, it is held for 30 minutes prior to homogenization and cooling. This process is also known as the low-temperature long-time (LTLT) method of pasteurization.

In the continuous process, a plate heat exchanger is used. This heat exchanger has three sections called (a) regeneration, (b) heating, and (c) cooling sections. In this process, the mix is heated to a minimum temperature of 79.9°C (175°F) and held for 25 seconds; 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 seconds. 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, these flow diversion devices sense this and send the mix to be repasteurized. 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 so that 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). One schematic of a continuous pasteurizer also called a high-temperature short-time (HTST) is shown in Figure 16.4.

HOMOGENIZATION

The process of homogenization has been referred to during the pasteurization step. 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. One micrometer is 1/25,000th of an inch, a size not visible to the naked eye. In order to homogenize the ice cream mix, all the fat must be in the liquid state. Therefore, homogenization is performed on hot mix. In an 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 13.8 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 (1,200 psi) first stage and 3.45 MPa (500 psi) second stage.

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.

FLAVORS

Flavor is the most important esthetic attribute of a food and ice cream is no different. Ice cream differs from other food products in that it has no preconditioning 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. You can smell ice cream and not be able to discern the flavor of the frozen product. The term *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. The flavor of ice cream can be treated analogous to the ubiquitous computer. Sensory evaluation involves an input in the form of stimulation. The brain is the microprocessor that transforms these sensations and the output is the reaction of like, dislike, and so forth. Because the subject has psychological and physiological variations, the response is not as reproducible as that of an instrument. Therefore, statistical techniques are employed to understand the consistency of reactions of the fictional instrument called the human brain. This chapter will not delve into the intricacies of sensory evaluation.

Flavor is an important attribute of a food. It is a sensory response that has three components: olfaction (odor/smell), gustation (taste), and tactual (mouthfeel). Ice cream is cold, creamy, refreshing, and sweet and releases aroma upon melting in the mouth. When the word “flavor” is used in everyday parlance we imply taste and olfaction. Taste compounds are sweet, salty, bitter, and sour. Generally, compounds imparting these tastes can be detected at levels of 0.01–0.5%. Olfactory compounds (smelly stuff) are volatile and have thresholds in the parts per million to parts per trillion range. Threshold is defined as the minimum concentration that at least 50% of the population can detect. Threshold of aroma compounds are 10–10 million times less than taste compounds.

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. These 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, ice and fat melt. Melting of these two constituents leads to the collapse of the air cell. Upon collapse of the air cell, fl vor 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-fl vored base. This base has the ability to acquire any characterizing fl vor added to it. This neutrality of fl vor also means that if the mix is mishandled it can easily absorb off-fl vors. The ice cream mix contains fat, MSNF, sweeteners, stabilizers, and emulsifier and in some instances other additives. The preferred source of fat is milk fat and the fl vor quality of cream and milk should be carefully monitored. If milk fat is improperly handled, off-fl vors can easily result. When the source of fat is other than milk, other fl vors may be present. Most fl vor compounds are fat soluble. MSNF sources contribute a slightly salty note but can also contribute to stale, caramelized, old ingredient fl vor notes. Off-fl vors 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 fl vors. Corn syrup solids and high fructose corn syrup solids are also used as sweeteners. These could contribute a syrupy fl vor and may mask the delicate fl vor notes of some other ingredients. Mix processed in batch processors is particularly prone to this syrupiness. Stabilizers rarely pose fl vor problems but by increasing the viscosity may slow the release of delicate fl vors. Emulsifier rarely pose fl vor problems unless they are old. Rotating stocks and inventory control can avoid these problems.

Determining how much fl voring to add is always important. Know what your customers like. There are regional preferences for fl vors and intensities of fl vors. As a rule of thumb, higher the fat content of the mix, more the fl voring required. Also, batch pasteurized mixes, especially if they contain egg yolk solids, require more fl voring than HTST pasteurized mixes. Always test the effectiveness of the added fl vor in the ice cream and not by judging the quality of fl vored mix.

Flavors are added in at least three different ways, namely (a) directly to the mix prior to freezing (e.g., vanilla, chocolate, mint), (b) immediately post-freezing (fruit pieces, nuts, candy, and confectionery pieces), or (c) postfreezing prior to packaging (ripples and variegates). Modern fl voring systems are complicated and may use all three of these modes

of fl voring in the same ice cream. The most popular fl vors are vanilla, chocolate, fruits, nuts, bakery goods, confectionery items, and ripples or variegates. Nearly 30% of the ice cream manufactured is vanilla.

Vanilla is a standardized food. It is the only fl voring substance to be so classified. According to the Food and Drug Administration, single-fold vanilla extract must contain 13.8 oz vanilla bean material in 70 proof alcohol. Ice cream made with pure vanilla extract is labeled Category I as vanilla ice cream. Ice cream fl vored 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-fl vored ice cream. The third type is called Category III and consists of any vanilla that is not Categories I and II. Such a product is labeled as artificial fl vored vanilla ice cream.

Vanilla is the bean of the only edible orchid *Vanilla planifolia* Andrews. The bean only grows around the equatorial belt. Mexico, Madagascar, India, Indonesia, The Comoros are all good growing areas for the vanilla orchid. The beans produced from the orchid are cured and shipped to countries where the fl vor is extracted using alcohol and water.

Chocolate is another popular fl vor. Chocolate is derived from the beans of a plant called *Theobroma cacao*. This plant also grows in the equatorial regions of the world and countries. Ghana, Ivory Coast, Brazil, Cameroon, Indonesia, Malaysia are all noted for the production of cocoa beans. 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 fl voring material is 10/12 cocoa for light, low and no fat ice creams whereas 22/24% cocoa is used for regular ice cream.

Fruit fl vors are popular and can be added as extracts, essences often with other natural fl vors (WONF). 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-fl vored 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 3 parts of fruit (3 plus 1 pack) or one part of sugar per 4 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 fl vor improved due to the heat treatment. Stabilized packs need not be refrigerated and some stabilized fruits may have a jam-like fl vor rather than that of the fresh fruit.

Nuts like pecans, almonds, walnuts, cashews, hazelnuts, peanuts, macadamia, and pistachios are also used to fl vor 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.

Ripples or variegates are a method of fl voring ice cream which incorporates unusual appearance and fl vor into ice cream. A good ripple is soft fl vorful 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: (a) freezer whipping and (b) 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 can have been of such varied fl vors as chocolate, marshmallow, peanut butter, butterscotch, caramel, fudge, raspberry, blueberry, or other fruits.

Candy and confectionery pieces have become popular fl voring materials in ice cream. Toffees and hard candies are popular fl vors. Hard candies have a moisture content of <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.

Baked pieces like cookies, cookie dough, cakes, pie crusts, and so forth, are also used as fl vorings. 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.

The final point about fl vorings is that it is the first bite or lick that hooks the consumer if the fl vor is good. Therefore, fl vor is one of the most important attributes of ice cream. When purchasing fl vors, do not be influenced by the cost per unit of the additive

but rather by the impact and quality of the impact that results from this ingredient. Calculate the cost of the fl voring on a use basis rather than the cost of the ingredient per se.

FREEZING AND HARDENING

When freezing of ice cream is discussed, it is important to remember that it concerns the creation of ice from water in the mix. Therefore, the only constituent of the mix being frozen is water. During the freezing process the equilibrium between water and ice is altered. Freezing is facilitated by the removal of heat from a substance. In the old salt and ice machine, used prior to mechanical refrigeration, ice served as the refrigerant and addition of salt lowered the freezing point of water. The brine extracts heat from the mix. The mix temperature is lowered and the brine temperature increases. Brine is not a good refrigerant. With the advent of mechanical refrigeration, the use of ice and salt for freezing ice cream was relegated to a hobby status.

Refrigeration

Sensible heat is the heat which when added or removed causes a change in temperature of the product. Sensible heat can be measured by observing the temperature of the substance. Latent heat (hidden heat) is the heat required to bring about a change of state. For example, to convert water to ice at 0°C (32°F) requires the removal of a large amount of heat and latent heat cannot be observed by temperature changes.

Mechanical refrigeration relies on fluids that have a low boiling point and a high latent heat of vaporization. In the vapor phase the refrigerant must be dense, nontoxic, low flammability, immiscible with oil, and low cost. Ammonia is the commonly used refrigerant in large installations.

Mechanical refrigeration units rely on four elements: (1) evaporator, (2) compressor, (3) condenser, and (4) expansion valve. The refrigerant circulates between these four elements and changes state from liquid to gas to liquid. In the evaporator the liquid refrigerant evaporates under reduced pressure and in doing so absorbs latent heat of vaporization and cools the medium being frozen. The other parts of the refrigeration recycle the refrigerant. Refrigerant vapor passes from the evaporator to the compressor where the pressure is increased. The high pressure vapor then passes to the condenser where it is condensed to a high pressure liquid. The liquid passes through an

expansion valve where pressure is reduced and the cycle starts again.

Freezing Ice Cream

This refrigeration cycle is responsible for freezing water in an ice cream mix. Latent heat of crystallization for water is 1 kcal/kg or 144 British Thermal Unit (BTU) per pound. The freezing point of a food is the temperature at which a minute crystal of ice exists with the surrounding water. However, before an ice crystal can form, a nucleus of water molecules must be present. Nucleation therefore precedes ice crystal formation. In the freezing of an ice cream mix the freezing point is determined by the amount and types

This is desirable in creating a smooth textured ice cream.

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 1 gallon (3.75 liters) of mix is converted to 2 gallons of ice cream (7.5 liters) we have effectively doubled the volume of the mix. This is termed 100% overrun. Overrun can be calculated on a volume basis or on a weight basis as follows:

On a volume basis

$$\text{Percent overrun} = \frac{\text{volume of ice cream made} - \text{volume of mix used}}{\text{volume of mix used}} \times 100$$

On a weight basis

$$\text{Percent overrun} = \frac{\text{weight of unit mix} - \text{weight of equal volume of ice cream}}{\text{weight of equal volume of ice cream}} \times 100$$

of solutes present. The important solutes are sugars and milk salts and any other dissolved low molecular weight materials. In this regard, it must be borne in mind that monosaccharides depress freezing point to a greater extent than disaccharides and salts depress freezing point 2–3 times greater than an equal concentration of sugars.

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.

Example 1 500 gallons of mix were used to produce 850 gallons of ice cream. The overrun would be calculated as follows: $\{(850-500)/500\} \times 100 = (350/500) \times 100 = 0.7 \times 100 = 70\%$.

Example 2 A mix weighs 8.9 lb. to a gallon and the finished ice cream weighs 4.5 lb. to a gallon. The overrun would be calculated as follows: $\{(8.9-4.5)/4.5\} \times 100 = (4.4/4.5) \times 100 = 0.978 \times 100 = 97.8\%$.

In practical ice cream operations, a target overrun is chosen for the product and then package weights are calculated. For example, it is desired to make 85% overrun ice cream and a gallon of mix weighs 9.1 lb. the gallon of 85% ice cream is calculated by the formula:

$85\% \text{ overrun} = \frac{9.1-x}{x} \times 100$ or $\frac{9.1}{1.85} = 4.92$ lb. From this calculation the weight of a half gallon of this ice cream can be set at 2.46 lb. and a quart of this ice cream should weigh 1.23 lb. and a pint of this ice cream should weigh 0.61 lb. or 9.8 oz.

Types of Ice Cream Freezers

Batch Freezer. 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 it in to 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 minutes. There is some skill to operating such a freezer and batch to batch variations are routine in such products.

Continuous Freezer. Continuous freezers are commonly used in larger ice cream manufacturing plants where more than 500 gallons (1875 liters) 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 be also automated. Also, continuous freezers make it possible to produce ice cream of different shapes through extrusion devices. Novelty extrusions such as sandwiches, pre-illed cones and cups, cakes, and so forth, 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 hori-

zontal 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 of the ice cream, and the overrun of the ice cream. Further these microprocessors can work in tandem with other downstream equipment such as ingredient feeders and packaging lines.

In continuous freezers the air for the overrun has very little effect in the freezing cylinder because it is compressed. In a freezer operating with 4 atm. 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 semifrozen 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.

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) 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 temperatures is lower than -7°C (19°F) cylinder pressures may have to be increased by 2–3 atm.

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 $3\text{--}4^{\circ}\text{C}$ ($\sim 40^{\circ}\text{F}$). Newer freezer designs make it possible to extrude ice cream at -18°C (0°F) creating some interesting and desirable texture characteristics.

The consistency of ice cream as it is drawn from the freezer is often referred to as “wet” or “dry” or “stiff.” The terms *dry* and *stiff* are used interchangeably in parts of the world. This consistency is influenced more by formulation than by any other factor. 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.

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) and exits at –5°C (23°F) and the refrigerant evaporating temperature is –5°C (23°F) or 2 psig pressure, a valid comparison can be made for ice cream throughput at 100% overrun. It is critical to have all conditions illustrated above 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.

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. 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 toward the center of the package. As more water gets converted to ice it acts as an insulator. Therefore, it takes 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 half gallon of ice cream reach –18°C (0°F) in 3 hours or less. In order to achieve the air temperature has to be at least –28.9°C to –34.4°C (–20 to –30°F). A larger package, such as 11.25 l (U.S. 3 gallons) 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 rooms can be batch or continuous. Batch hardening rooms consist of a very cold room with the ability to move large volumes of air about in this room. The products should arrange in such a manner that this cold air is able to sweep all surfaces of the packaged ice cream. This requires air spaces all around the cartons of ice cream. In a continuous hardening system the ice cream package traverses a box in which cold air is circulated. The ice cream enters this box on a conveyor belt at one end of the box and is conveyed in a repeating zigzag manner back and forth until the required residence time is attained. Then the hardened ice cream exits from this hardener and can be stored. Continuous hardening systems are called hardening tunnels.

Hardening apparatus configuration can be a room, tunnels, spiral tunnels, straight through tunnels, contact plate freezers, and special tunnels used in novelty manufacture.

In all hardening systems, frost builds up in the room over time. In humid environments, this process may be quicker than in arid environments. The room or tunnel has to be defrosted. Avoiding or minimizing frost build up is desirable. It is important to keep the evaporators and other parts of the room or tunnel frost free. Defrosting entails raising the temperature enough to melt and remove the frost. It consumes energy to defrost and additional energy is required to cool the room or tunnel back down to operating temperatures.

Hardened ice cream is stored at –28°C (–20°F) prior to distribution. 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, and so forth.

PACKAGING

A good package must contain the product, protect it, provide convenience, and provide information on the product to the consumer. Food packages provide protection against physical, chemical, and biological

damage. It also provides information useful to the consumer, for example, ingredient label, nutritional label, net contents, serving suggestion, and methods of preparing the product. Besides these attributes, a good food package keeps the food at nearly the same quality as when it was manufactured. During distribution packages are subjected to physical abuses such as shocks, vibrations, compression, and in the case of ice cream and frozen desserts, heat shock.

For frozen dessert packaging three main factors have to be considered. First, the package has to protect against temperature fluctuations photooxidation, dehydration, and odor transmittance. Second, it has to take into consideration distribution-related factors such as package integrity, thermal shock, and cube efficiency. Third municipal solid waste management factors have also to be considered.

In a consumer study performed prior to the passing of the Nutritional Labeling and Education Act (NLEA) 90% of the consumers polled wanted tamper evident packaging and nutritional information (particularly calories and sodium contents). The latter is now mandated in a defined format by NLEA and has adequately addressed that concern. Almost 80% of the consumers desired ice cream packages that hold up better and not get soggy. Also noted were resealable rectangles and cartons that shrank as product was used.

Ice cream was packaged in the 1920s in paperboard tapered pails with handles with easy open tabs and was of small size for total consumption. In the 1930s, various configurations of packages which were small enough to fit into the ice cube tray compartments of home refrigerators paperboard containers appeared. Such containers were no larger than a quart and were reclosable tab lids. In the 1940s and 1950s, the package size increased to half gallon with appropriate strength to be filled automatically and had reclosable tabs at either end. In this era round containers were positioned as premium products. In the 1960s through 1970s, plastic rounds, two piece cartons coated for higher quality graphics, zippered tab opening and hooded hinged lids were introduced. In the decades of 1980s through 1990s, tamper evident shrink film and bands, two piece containers in a wide range of materials, transparent and translucent packages, membranes inside packages to reseal and rectangular packages with rounded corners were some of the notable developments. These evolutionary patterns follow the penetration of domestic refrigerators and the changes in sociological and demographic factors.

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.

As far as protecting the product from heat shock goes, there are a number of variables which have an impact on this. The cold chain which consists of the steps involved in moving ice cream from the factory to the consumer is a major variable. The number of steps involved may be as simple as moving the ice cream from the hardening room to point of sale as may be the case in a small shop to as complex as moving ice cream from the factory to a factory warehouse, then to retailer's distribution center and from there to the store freezer, display freezer, transport home, and finally consumption. Transportation is involved in this chain and distance and altitude of travel may also play a role. Because of these variables it is often hard to arrive at a reliable sell-by date for ice cream. The problems may be exacerbated in the case of novelties because generally these products are smaller in volume and therefore more susceptible to melting and refreezing. In many instances manufacturers do not know the extent of temperature variation in their distribution system.

In a Finnish study conducted in 1984 compared different package types stored at different temperatures in both chest and upright freezer cabinets. Product acceptability was evaluated using a sensory panel (Table 16.8).

STORAGE AND DISTRIBUTION

Frozen and hardened product is stored and often distributed prior to the enjoyment by the end consumer. The intermediate steps involved in storage vary depending upon scale of manufacture, market share, point of sale, and consumer preferences. In the simplest case of a retail ice cream manufacturer, the product is made fresh in the store and sold very soon after manufacture and this requires relatively few controls. In an extreme case, product made on one of the coasts of the United States is transported for sale to the opposite coast. Here, time, transport conditions, altitude,

Table 16.8. Effects of Packaging Materials, Display Cabinet Style, and Temperature of Storage on Shelf Life of Ice Cream

Package Material	Time Until Product Is Not Fit for Sale (Weeks)			
	Vertical Cabinet		Open Display	
	−12°C	−15°C	−18°C	−15°C
Cartonboard packages				
Hot melt coated	8–10	11–16	22	Nd
PE-coated	6–8	11–16	13–17	10
Al Foil laminated	Nd	Nd	Nd	16–18
Plastic packages				
PS-box	16	16–22	>30	Nd
HDPE-box	16	19	22–26	Nd

Abbreviations: PE, polyethylene; PS, polystyrene; HDPE, high density polyethylene; Nd, not determined.

temperature, humidity, refrigeration conditions, and so forth, have to be carefully controlled in a manner that the frozen dessert maintains its quality when the consumer eats the product.

Ice cream is unique in that it is the only product that is consumed in the frozen state. Therefore, once it is manufactured it has to be stored, transported, distributed, and sold in the frozen state. In the United States, frozen foods are distributed in a separate chain than ice cream is because the cold chain for frozen foods is −18°C (0°F) and is inadequate for ice cream. Ice cream cold chain maintains −23°C (−10°F). The distribution chain is called the cold chain and varies from manufacturer to manufacturer. Regardless of the variations one thing is certain. The cold chain is imperfect. This imperfection affects the quality of the product at the point of purchase and impacts consumer satisfaction. Factors affecting the shelf life of ice cream are manufacturing procedures, warehouse equipment, warehouse handling practices, transportation, storage at retail premises, retail display equipment, and retail handling practices.

Manufacturing Procedures

Mix formulation, the adequacy, functionality, and quality of ingredients are all important parameters that receive careful attention. This is followed by proper blending to achieve intended functionalities of the added ingredients. Next, pasteurization and homogenization, freezing, packaging, and hardening have also to be carefully monitored and controlled. Packaging and outer case for products should be of good quality to prevent contamination, insure

integrity of product during normal storage and transportation, minimize dehydration, and also but not the least package coding should be adequate for effective identification. Outer coding is useful for proper stock rotation and phrases such as “store at −29°C (−20°F) or colder should appear on outer cases. Lot pallet or unit load identity is useful in proper stock rotation while maintaining lot identity.

Warehouse Equipment

Warehouse should be of adequate capacity, suitably refrigerated to maintain a steady air temperature of −29°C under peak loading conditions and maximum ambient temperature. Storage areas must be equipped with accurate temperature recording devices. Daily checks of temperatures of each area in the warehouse should be maintained and recorded. Automated recorders are preferable and data should be retained for a 2-year period. Warehouse operator should record the product temperature of each lot of product received and should accept custody only in accordance with good commercial practice. Records for arrival lot temperatures should be retained for a period of 1 year.

If products are received above −29°C, the operator should immediately notify the owner or consignee and request instructions for special handling. Product received above −29°C had incurred heat shock and may pose quality problems. Before placing the shipment in storage it should be code marked for effective identification. Every effort should be made to minimize exposure to elevated temperatures and humidity conditions.

During defrosting, operations cover products beneath areas of accumulated frost. Products going in to the staging areas for order assembly must be moved out of the area promptly unless the staging area is maintained at -29°C . As many of the operations should be carried out in the cold if possible. Allow the bottom of the stack to have air passage and leave adequate room between stacks for proper air circulation.

Transportation

Vehicles used for transporting ice cream products should be clean, free of dirt, offensive odors, debris, and so forth. They should also be insulated and equipped with adequate refrigeration. The vehicle must have tight fitting doors without air leaks and should be precooled for 25 minutes prior to loading. Transport vehicles must also have accurate, visible, and readable temperature recording devices. The thermostat on the truck should be set to maintain an air temperature of -29°C with proper airflow or circulation. During loading and unloading operations the refrigeration unit must be off.

Storage on Retail Premises

Storage on retail premises must have adequate refrigeration capacity to maintain a product temperature of -29°C and of sufficient size to maintain proper stock control and rotation. Storage facility must have adequate circulation of cold air all around the products. Storage facilities must be equipped with an accurate, readable temperature recording device which is easy to calibrate. Facility should be defrosted regularly as necessary to maintain refrigeration efficiency.

Retail Display Equipment

Display cabinets should be capable of maintaining -29°C and should be situated away from drafts, direct sun, heat-producing equipment, or any other factor likely to reduce its efficiency. The display case should have a calibrated easy to read thermometer at a location representative of the average temperature of the cabinet. Display cabinets should have a properly marked load limit on the cabinet walls. To facilitate air circulation, cabinets should have sufficient dividers, separators, and grids. Cabinets must be defrosted when necessary to assure proper operation. It should be kept free of debris, signs, and tags that deflect refrigerated airflow.

Retail Handling Practices

Ice cream and frozen novelties should be delivered in a frozen condition at -29°C . Warmer products should be rejected or if accepted examined for quality and put up for quick sale. Once unloaded at the retail end, the products must be moved quickly to the freezer. Inventory must be rotated on a first-in first-out basis. Any cases not bearing a code or date should be dated upon receipt. When loading ice cream into display cabinets rotate inventory already in the cabinet. New products should be placed beneath the existing stock. Items should not be placed outside of the designated load limit lines and care should be taken not to block airflow. Store personnel should be aware of maintenance and sanitary upkeep of the freezers.

For the operation of efficient and optimal cold chain educational programs for all personnel handling ice cream must be implemented. This will insure that the customers receive products in the best possible condition.

Heat Shock

Heat shock is a term used to describe temperature fluctuation that occur during the storage and distribution of ice cream. The aim is to minimize heat shock and its deleterious effects. Rise and fall of temperatures affect the water-ice equilibrium in frozen desserts. During the freezing and hardening of ice cream extreme care is taken to create the largest number of small ice crystals. Ice crystal nucleation is initiated in the ice cream freezer where the objective is to freeze approximately 50% of the water in the formulation as quickly as possible. The remainder of water that can be frozen changes state in the hardening process. No new ice nuclei are created in the hardening process. Following the laws of thermodynamics large ice crystals grow at the expense of small ice crystals. Every time the temperature rises some of the ice melts and when the temperature goes down it refreezes. The refreezing takes place on existing ice nuclei and thus the number of small ice crystals decreases while that of larger ice crystals increases. The amount of ice thawing and water refreezing is dependent on the extent of temperature fluctuation and the frequency of this fluctuation. When ice crystal sizes reach $150\ \mu\text{m}$ the coarseness begins to get apparent to the tongue. Other effects of heat shock are the loss of shape and eye appeal of novelties and if persistent loss of market share for the manufacturer.

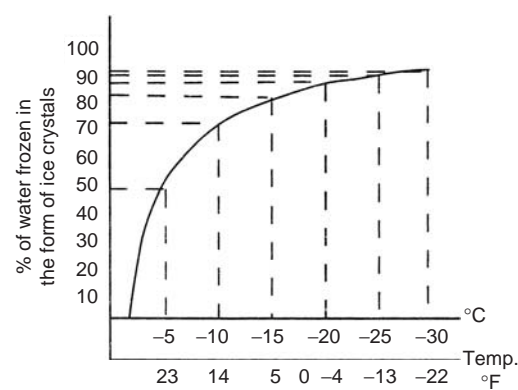


Figure 16.5. Freezing/thawing curve for a typical ice cream mix.

Depending upon the solute composition of the ice cream mix a freezing and thawing curve for that mix can be constructed (Fig. 16.5). Such a curve can provide the amount of ice and water changing state for

define temperature change. At very low temperatures relatively little water/ice change state.

As the temperature warms more water/ice change state. If a greater amount of water/ice changes state the shelf life of the product will shorten considerably when compared to a much smaller change in temperature. An example of the field data of temperature changes during storage and distribution of ice cream is provided (Fig. 16.6).

In this illustration, the ice cream was “all natural” with no added stabilizers or emulsifiers. The overrun was approximately 100%. Whenever the product was in transit the temperature fluctuation were large. When the warehouse was under the management of the manufacturer product handling was good. Whenever warehouse control was relinquished by the manufacturer the temperature fluctuation increased. In this example the product was coarse within 90 days.

Display cabinets at the retail point of sale are also notorious for causing heat shock to the product. The common types of display cabinets are open top merchandisers (also known as coffin style) and upright with glass doors. Consumers also keep doors open

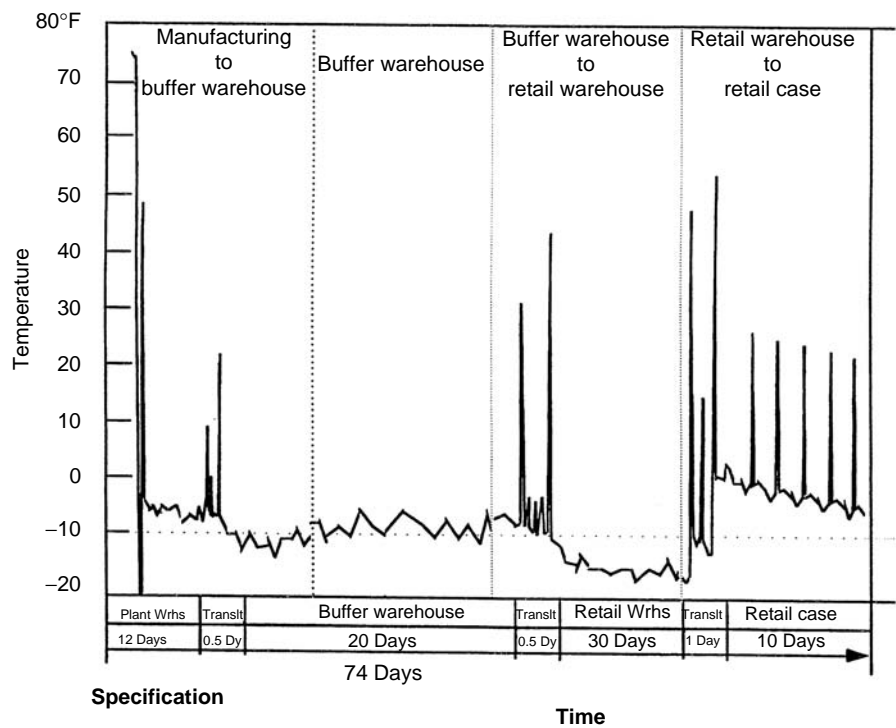


Figure 16.6. Temperature fluctuations in a commercial ice cream as it passed through the cold chain.

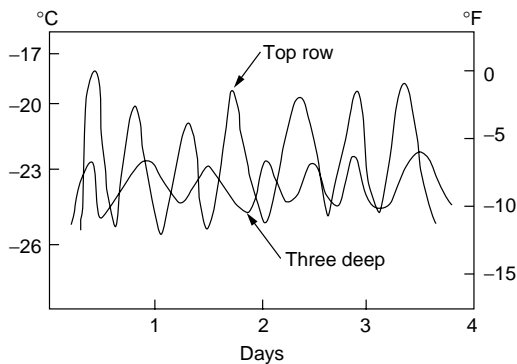


Figure 16.7. Temperature of ice cream in an open-faced cabinet which defrosts two times a day. Rectangular cartons of ice cream were placed three deep in this style of cabinet.

while making their choices leading to frost build up and necessitating frequent defrost cycles, which in turns adds heat shock. A survey of ice cream manufacturers in the United States revealed that physical defects were more frequent than flavor defects. Customers reacted unfavorably to physical defects. Defects occurring often were reported to be icy texture, shrinkage, gummy/sticky texture, and evidence of previous melt. Customers were more tolerant of icy or coarseness than other physical defects. These defects objectionable to the consumers are all a result of heat shock.

If rectangular 1.88 liters (U.S. 0.5 gallon) containers of ice cream are placed in an open top merchandiser packed three deep and the merchandiser undergoes two defrost cycles per 24 hours the temperature fluctuation are as depicted in Figure 16.7.

As expected, the carton on top undergoes the greatest fluctuation and the cartons on the bottom of the pile undergo the least fluctuations. In such cartons the product contained in the outside 2 cm represents 60% of the total ice cream in the container while the center 2 cm portion represents only about 15% of the volume of the container. In approximately 4 days the amount of water that is formed and converted to ice is equivalent to the total water that was originally present in the mix.

NUTRITIONAL VALUE OF ICE CREAM

Table 16.9 gives an example of nutritional label of ice cream. It is evident that ice cream furnishes vital

constituents and nutrients of milk, such as protein, calcium, and other minerals, as well as vitamins.

MANUFACTURE OF ICE CREAM NOVELTIES

Novelties are products that have unique characteristics such as shape, color, and packaging. Typically, novelties offer convenience, portion control (individual serving), unique forms (shape, size, color, flavor, and package). These are value-added products conducive to impulse purchase. Novelties can be classified as sticks, bars, slabs, bite size, cups, and cones. These products can be molded, that is, shaped by pouring and freezing mix in a mold or extruded. The products can be ice cream, water ice, sherbet, or a combination of these.

MOLDED NOVELTIES

Novelties that acquire their shape from a mold or form are called molded novelty items. In such products a mold is filled with a mix or ice cream and then frozen further. The product is then demolded (removed from the mold) and enrobed in or coated. Most often molded products are stick products. Sticks are inserted into the semifrozen product in a mold and then the freezing process is completed. When such a product is demolded the stick serves as a means of holding the product while eating. Equipment that manufactures molded novelties can be straightline or rotary machines. In a straightline machine there is a linear array of molds. Molds can be easily changed. Straightline machines occupy more floor space, are easier to clean and sanitize but the water use is greater as is the waste generation. In rotary machines molds are arranged radially on a large circular wheel and this configuration uses less floor space than straightline machines. There is less product lost in the molds but cleaning is more difficult and mold changes are time-consuming. Molds are made of special alloys that have good heat transfer capacity, mechanical durability, resistance to brine, and resistant to corrosion from cleaning and sanitizing solutions. Molds can also be made from plastics which have particular advantages in three-dimensional effects. Generally molds are 0.22 mm (0.75 in.) thick and volumes of molds vary from 50 mL (1.8 fl oz) to 75 mL (2.8 fl oz). Molds are held in place on an arm with several molds wide on a rotating conveyor belt. In a straightline machine the molds traverse from one end of a cold brine trough to the other. Various operations are

Table 16.9. Nutritive Value of One Serving (1/2 cup, 4.4 fl. Oz, 120 mL or Approx. 66 g) of Ice Cream

	Vanilla		Vanilla		Vanilla		Orange Sherbet,	
	Ice Cream, 4% Fat	% Daily Value ^a	Ice Cream, 10% Fat	% Daily Value ^a	Ice Cream, 16% Fat	% Daily Value ^a	2% Fat	% Daily Value ^a
Total calories (kcal)	123.4		133.6		174.7		135.8	
Calories from fat (kcal)	34.1		64.0		106.6		17.3	
Total fat (g)	3.8	6	7.1	11	11.8	18	1.9	3
Saturated fat (g)	2.4	12	4.4	22	7.4	37	1.2	6
Cholesterol (mg)	12.2	4	29.5	10	43.8	15	7.1	2
Sodium (mg)	70.2	3	57.6	2	54.1	2	44.4	2
Total carbohydrate (g)	19.5	6	15.7	5	16	5	29.5	10
Dietary fiber (g)	0	0	0	0	0	0	0	0
Sugars (g)	18.4		14.8		15.1		27.8	
Protein (g)	3.5	7	2.4	5	2.1	4	1.1	2
Vitamin A (IU)	143.4	2	269.3	6	448.4	8	93.1	0
Vitamin C (mg)	0.5	0	0.4	0	0.3	0	1.9	4
Calcium (mg)	118.3	10	87.2	8	75.6	8	52	6
Iron (mg)	0.1	0	0.1	0	0.1	0	0.2	0

^a% Daily value is based on 2,000 calorie diet.Source: Nutritive Value of Foods, Home and Garden Bulletin Number 72, Oct. 2002. www.nal.usda.gov/fnic/foodcomp.

conducted in stages during this travel from end to end. There are also rotary machines in which the molds rotate around a drum containing cold brine and various operations can be staged at different points in this rotation. In either case the novelty making process starts with charging the molds with ice cream and ends with the demolded product transferred to packaging stations.

The steps in molded novelty manufacture involve filling the mold with partially frozen mix. The filled molds are frozen to a consistency that can support a stick upright and then the stick is inserted. Freezing proceeds further until a stiff consistency is obtained. Then the product is demolded. Demolding takes place by passing the molds through a warm brine zone where the outer layer of the product is softened enough so that the product can be removed from the mold. The product is removed from the mold by lifting it from the mold. The removed product can then be dipped in water, chocolate coating, and so forth. The coated products can then be dipped in nuts, candy, sprinkles, and so forth, prior to packaging. Some coatings may require a refreezing of the product. This refreezing can be accomplished by immersion in liquid nitrogen. This step may be repeated several times to build up a thick layer of the coating. The packaged products are put into multi-packs or cartons and sent for hardening. The terms used in molded novelty manufacture are as follows: "wide" refers to the number of individual molds in a row of the machine, for example, 8 wide means 8 molds in one row or 12 wide means 12 molds in a row. "Strokes" means number of pieces produced in one synchronous movement. It is a measurement of speed. Typical strokes are 16–24 per minute. So an 8-wide machine operating at 16 strokes per minute produces 128 pieces per minute or 7680 pieces per hour.

Several variations can be made in the manufacturing process. For example, one third of the mold can be filled with one flavor, partially frozen and a second flavor added to make two thirds of the volume and finally another third of a different flavor can be layered on top prior to stick insertion. A second instance of variation is what is called shell and core freezing. In this type of stick novelty the outside has a different flavor and product than the inside. This is achieved by filling the mold with one flavor and allowing that flavor to freeze along the inner edges of the mold; the unfrozen mix is aspirated from the mold followed by the filling of a second flavor or type of product. Freezing, sticking, demolding, and packaging then continues after these stages. By controlling

Table 16.10. Comparisons Between Molded and Extrusion Processes

Characteristic	Molded	Extruded
Ice cream temperature	–3 to –4°C	–6 to –7°C
% Water frozen	25–35	55–60
Texture from freezer	Fluid	Stiff
Flow	Intermittent	Continuous
Shape defined by	Mold	Orifice
Texture after hardening	Coarse	Smooth

the extent of product frozen in the mold prior to aspiration of the unfrozen mix different thicknesses of flavors can be achieved.

EXTRUDED NOVELTIES

In the manufacture of this type of novelty ice is extruded through an orifice and then separated into individual portions (frequently by a heated wire). Extruded novelties can be stick or stickless products. Sticks are inserted immediately after extrusion and prior to cutting. Extrusion can be horizontal or vertical. Horizontal extrusion is also called band extrusion. Co-extrusion is also possible where more than one type of ice cream is extruded through the orifice. Cups, cones, and other filled products can be made by extrusion. Extruded portions are then hardened in a spiral freezer. Because the volume of the extrudates is small hardening can be achieved rapidly. Hardened products can then be coated/enrobed and packaged. The types of extruded novelties include cups, cones, sandwiches, cakes, bite size miniatures (e.g., bonbons), and candy bars style products. Extruded items can be decorated during extrusion. A small orifice in the shape of a star is often used to create a rosette and up to eight such devices can be used simultaneously to decorate an ice cream cake. The true expertise in the manufacture of molded novelties is in the design of the extrusion nozzles and in conceiving unique shapes or forms. Comparisons of molded and extruded novelties are described (Table 16.10).

RECENT DEVELOPMENTS

As a result of research conducted by scientists at the Federal Institute of Technology in Zurich, Switzerland, a new way of freezing ice cream to much lower temperatures has been achieved. A piece of equipment called ULTICE freezes and whips ice cream mix to temperatures of –18°C (0°F). A twofold benefit of

such freezing is the improvement in eating quality and extension of shelf life. Extension of shelf life is achieved by having a greater number of smaller ice crystals when compared to conventional ice cream. Further the orientation of the larger number of smaller ice crystals is also thought to be important. Improvement in eating quality results from air cell and fat agglomeration effects. Lower fat ice cream (6% fat) can taste as good as 12% fat ice cream conventionally frozen. There is increased shape retention of ice cream because of the increase in stiffness of the extrusion at -18°C . Ice cream mix is frozen in a conventional ice cream freezer where the exit temperature is -4.4 to -5.5°C (22 – 24°F) and approximately 50% of the water is frozen into small ice crystals. This output then enters a twin screw extruder exiting at temperatures as low as -15°C (5°F) or lower.

Potential advantages of such a freezing technique involve cost reduction. The ability of reducing fat in ice cream mix without altering the eating quality of the final product may allow for reducing cost of the mix. Because more water is frozen in the freezer, hardening time can be reduced resulting in saving energy. In novelty manufacture it may be possible to avoid hardening all together (Bruce Tharp, personal communications). Products are available for distribution in a time shorter than conventionally frozen products.

Some alternative approaches to ULTICE have also been attempted by equipment manufacturers. Tetra Hoyer, Gram, and Cherry Burrell have modified extruders that do not require a twin-screw extruder. Tetra Hoyer's freezer freezes the conventionally frozen ice cream at -5°C (23°F) and passes through again in a freezer barrel providing an outlet temperature of -9 to -10°C (14 – 16°F). Cherry Burrell recirculates ice cream continuously at slow speeds along with pre-aeration. It is thought that smaller air cells and fat sparing effects are achieved in such a system. Overrun control is facilitated and accurately controlling overrun is also achieved. Gram's freezer includes pre-aeration by a method that minimizes shear. The dasher speed is also reduced when compared with conventional freezers and exit temperatures of -11°C (12°F).

This chapter has provided an overview of the manufacture of ice cream and other frozen desserts. There obviously are many more facets to this complex process. Changes occurring at a molecular level, the physicochemical basis for structure formation, sensory evaluation methods, proper cleaning and sanitation procedures and new developing trends in the

industry are not discussed in this chapter. Their omission here must not be misconstrued as the lack of importance of these topics. Additionally, the demand for ice cream and frozen desserts is increasing globally. With this global demand newer flavors, nutritional concerns, and cost effective manufacture and delivery of these tasty treats gains greater importance.

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Puddings and Dairy-Based Desserts

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INTRODUCTION

Milk-based puddings and custards have been consumed around the world for a long time. In the United Kingdom and certain Commonwealth countries, the word pudding is synonymous with dessert. Pudding word is probably derived from the French *boudin* with roots from the Latin *botellus* which means small

sausage referring to encased meats used in medieval puddings (Wikipedia, 2007). In general, pudding is a semisolid food obtained by cooking, baking, or steaming a cereal, flour, or batter with eggs, suet, blood, or milk. It may be used as a snack or dessert item or may form the filling portion of a pie or tart.

In the United States, pudding and dairy desserts constitute thickened and set product made by adding sugar, varieties of starch, rice or rice powder, tapioca granules, gelatin, and seaweed extracts such as alginates, carrageenan, and other hydrocolloids, tapioca, or eggs to milk or skim milk. Crushed nuts and dry fruits may also be incorporated to add the variety of texture and flavor.

Dairy desserts and puddings are divided into (1) ice cream and frozen desserts, (2) refrigerated products, and (3) shelf-stable products. Chapter 16 discusses ice cream and frozen desserts. This chapter deals with the refrigerated and shelf-stable category that includes pudding, custard, mousse, whipped cream, crème brûlée, cheesecake, cream pie, and tiramisu. The final texture in most dairy desserts is derived from the interaction of milk casein with carrageenan and viscosity-generating activity of modified starch. The texture varies from soft, creamy, and spoonable to gelled and firm. Flan and some puddings may be firm enough to be molded in a packaging cup with a sauce or syrup at the bottom. After removal from the cup and retrieval in a dessert plate, it is consumed with the sauce flowing down from the top. Generally, these products are neutral in pH. Occasionally, they are directly acidified or cultured. The custards and puddings may be prepared or baked in a piecrust to make pies such as tart, cheesecake, key lime pie, or cream pie. In general, these dairy desserts do not have

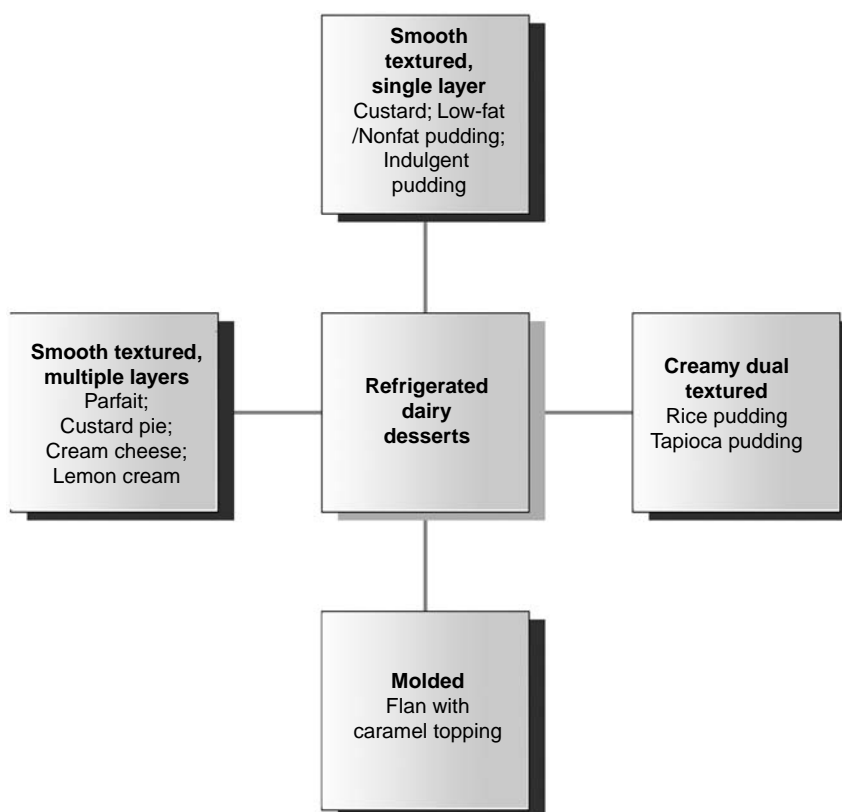


Figure 17.1. Major classification of puddings and dairy desserts.

a standard of identity define by the U.S. Food and Drug Administration (FDA).

Originally, puddings were prepared in the kitchen from scratch using raw ingredients. Dry mixes containing starch, flavor, and color provided more convenience of use at home. The advent of shelf-stable, ready-to-eat puddings in single service containers gives the consumer convenience and portability.

Dessert puddings include bread pudding, carrot pudding, chocolate pudding, plum pudding (Christmas pudding), fruit pudding, rice pudding, tapioca pudding, date and toffee pudding, pie fillings blancmange, custard, junket, mango pudding, parfait, mousse, vanilla, butterscotch, and pistachio pudding. Puddings in frozen form are exemplified by frozen custard and pudding pops.

MARKET VALUE

The market for “chilled” dairy desserts is significant in European countries. However, in the United States,

pudding is not perceived by the consumer as healthy as yogurt. In reality, pudding is also a dairy category that is wholesome, nutritious food, low in fat, and high in calcium content (Smith, 2003). In 2004, the sales of refrigerated pudding/mousse/gelatin/parfait brands were US\$570.4 million with a change of -0.1% from previous year. Total sales for shelf-stable pudding/gelatin brands were US\$283.6 million and the sales growth versus previous year was 7.7% (AllBusiness, 2007).

TYPES OF PUDDINGS AND DAIRY DESSERTS

Various types of puddings and dairy desserts on the market are classified in Figure 17.1. The broad classification shows some examples of common names of desserts and snacks. Recent trends include more technically sophisticated products. The examples are as follows:

- Layered mousses,
- Pudding with swirls,
- Vertically and horizontally layered products,
- Combining mousses, gelatin desserts, flans cakes, and puddings,
- Also, healthy and indulgence products are appearing in the market.

INGREDIENTS

The procurement of all ingredients should be based upon specification and standards which are checked and maintained with a systematic sampling and testing program by the quality control laboratory.

MILK

The manufacture of pudding starts with judicious selection of raw materials, accurate formulation, and processing of pudding mix.

Various dairy raw materials for formulating pudding mixes consist of milk, reduced fat milk, and skim milk. Occasionally, nonfat dry milk (NFDM), cream, or condensed milk may be used. Milk constitutes the basic raw material for all dairy ingredients. It is emphasized that all dairy raw materials should be selected for high bacteriological quality for securing best flavor potential in pudding. Milk should come from healthy cows that are fed wholesome feed and kept in clean surroundings. The flavor, consistency, and acid production are adversely affected by using milk from cows with infected udders (mastitis), general sickness, or in early or late stages of lactation, including milk containing high bacterial count, abnormal somatic cell count, and antibiotics, disinfectants, or sanitizers.

The microbiological analysis of milk for bacterial count, coliform count, and mold and yeast count should be done by standard procedures. In general, the methods are defined in the publications of American Public Health Association (2004) and AOAC International (2003). Basic information on the procedures for milk is presented in the publication by Marshall (2006) and in Chapter 23 of this book. Standard plate count (SPC) and coliform tests should be performed on each load of milk. A yeast and mold test should be done on a random basis. Although coliform, yeast, and mold are readily destroyed by heat treatment, their presence along with significant number of bacteria is an indication that the milk was handled in unclean equipment, or held under warm conditions. When milk comes into contact with unclean

surroundings, it could be contaminated with certain microorganisms secreting relatively heat-stable proteolytic enzymes capable of attacking milk proteins leading to undesirable flavors and weak body in puddings.

Also, if the milk has been contaminated with a high bacterial load, it is possible that these bacteria might be psychrophilic or psychrotrophic organisms. These organisms grow well in cold conditions. They grow slowly in milk held at 3°C, but growth may be rapid as the temperature rises to 10°C or higher. Although psychrophiles are readily destroyed by pasteurization temperature, if allowed to grow in significant numbers, they can produce heat-stable proteolytic enzymes which would degrade the protein. Again, the protein degradation results in weak pudding sets and possible off-flavors. There is a procedure for detecting psychrophiles outlined in the Standard Methods for Analysis of Dairy Products (American Public Health Association, 2004). However, a quicker modification version can be performed by incubating pour plates at 21°C for 25 hours.

Pudding mix composition regarding milk fat and milk solids-not-fat is generally standardized from whole, partially defatted milk, NFDM, and/or condensed skim milk, and cream. The chemical composition of dairy ingredients commonly used in pudding manufacture is given in Table 17.1. Formulating pudding mix to desired fat and milk solids-not-fat by the use of these ingredients can be easily accomplished by appropriate software programs.

CONCENTRATED MILK PRODUCTS

Removal of a significant portion of water from milk yields a series of dairy ingredients (Chandan, 1997). Consequently, these ingredients offer tangible savings in costs associated with storage capacity, handling, packaging, and transportation. A concentrated dairy ingredient used in large pudding manufacturing plants is NFDM and in some cases condensed skim milk.

Condensed Skim Milk

Condensed skim milk process begins with liquid raw whole milk, which is stored at the processing plant at temperatures below 7°C. Raw whole milk has a variable fat content and is separated into cream and the nonfat milk using a centrifugal separator. This separation step facilitates standardization of the fat content prior to further processing. Centrifugal separators used also serve to further clarify the milk. The

Table 17.1. Typical Chemical Composition of Dairy Ingredients Used in Formulating Pudding Mix

Ingredient	Total Solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Whole milk	12.6	3.5	3.5	4.9	0.7
Skim milk	9.5	0.1	3.6	5.1	0.7
Heavy cream	42.7	36.8	2.2	3.2	0.5
Condensed skim milk	40	0.4	39.6	10.8	2.22
NFDM	96.5	0.8	35.9	52.3	8.0
WPC-34	96.5	4.0	34.5	51.0	7.0
WPC-50	96.5	4.0	50.5	36.0	6.0
WPC-80	96.5	6.0	80.5	5.0	5.0
WPC isolate	96.5	0.5	93.0	1.0	2.0

WPC, whey protein concentrate; NFDM, nonfat dry milk.

Adapted from Chandan (1997).

skim milk is pasteurized (high-temperature, short-time) by heating to at least 71.7°C, and holding at or above this temperature for at least 15 seconds. In its production, the original skim milk volume is reduced to about one-third to yield about 35–40% solids in the final product using energy-efficient multi-effect evaporators that operate in high vacuum condition to boil off water at moderate temperatures of 46–55°C. The condensed milk is continuously separated from water vapor to achieve desirable concentration of milk solids. It is cooled to 4°C or below and pumped to insulated trucks for transportation to pudding plants. The cream produced from the separator is high-temperature short-time pasteurized, cooled, and transferred to cream storage tanks for use as a manufacturing ingredient.

Nonfat Dry Milk

Nonfat dry milk is made from condensed skim milk. Spray drying involves atomizing condensed milk into a hot air stream 180–200°C. The atomizer may be either a pressure nozzle or a centrifugal disc. By controlling the size of the droplets, the air temperature, and the airflow, it is possible to evaporate almost all the moisture while exposing the solids to relatively low temperatures. Spray drying yields concentrated and dry milk ingredients with excellent solubility, flavor, and color. This is the most common procedure for manufacturing concentrated and dry milk ingredients.

The spray-drying process is typically a two-stage process that involves the spray dryer at the first stage with a static fluid bed integrated in the base of the drying chamber. The second stage is an external vibrating fluid bed. The product is moved through the

two-stage process quickly to prevent overheating of the powder. The powder leaves the dryer and enters a system of cyclones that simultaneously cools it.

Heat treatment affects the functional properties of NFDM, so the temperature and time combinations can vary widely depending on the required properties. The milk heat treatment determines the kind of powder produced. For NFDM, produced by a “low-heat” method, the milk is simply pasteurized and no preheating is done. However, heat treatment for a “high-heat” method requires heating milk to 85–88°C for 15–30 minutes in addition to pasteurization. Heat treatment in between pasteurization and “high-heat” treatment yields “medium-heat” powder.

The extent of heat treatment can be measured by the whey protein nitrogen index, which measures the amount of undenatured whey protein. For use in pudding manufacture, only low-heat (whey protein nitrogen index ≥ 6.0 mg/g) NFDM is used.

Cream

Cream is used to standardize fat content of pudding mix, especially in case of indulgent versions of puddings. It is prepared from milk by centrifugal separation. Cream used as an ingredient contains 36–40% fat. It is pasteurized and should be stored under refrigeration. Some plants may freeze it to extend shelf life.

Whey Solids

The addition of whey solids in the form of sweet whey or acid whey to replace NFDM in pudding mix should be avoided. Whey solids will contribute to the total solids content of the mix; however, because

of lower protein content (13–15%) for whey solids as compared to 35–36% for NFDM solids, and lower protein functionality in terms of water binding capacity, the addition of whey solids can be detrimental to the consistency and firmness of the body of pudding.

On the contrary, whey protein concentrates (WPC), in relatively undenatured form, furnish excellent water-binding properties and may be a useful functional protein source in pudding mix. WPC are products derived from cheese whey by removal of minerals and lactose. The process of protein concentration utilizes membrane filtration (ultra-filtration which utilizes a semi-permeable membrane of appropriate pore size to retain large protein molecules while letting small molecules consisting of water, lactose, minerals, small peptides, and amino acids to selectively go into the permeate. On a dry basis, the WPC contains 34, 50, or 80% protein and whey protein isolate contains at least 92% protein. In addition, WPC-80 is available as gel type, which is designed to generate more viscosity in liquid foods. WPC-34 is suitable for use in pudding formulation. Since whey protein isolate and WPC-80 contain low levels of lactose, they are important ingredients in the formulation of low-carbohydrate pudding. Partial replacement of dry milk with WPC-34 allows the pudding processor may reduce the ingredient cost and at the same time provides unique functional properties including desirable nutrients, namely, high-quality whey proteins and calcium. WPC helps in heat-set gelation. Whey protein gets denatured by the heat treatment used in pudding mix preparation. The denatured protein has desirable water binding and adhesion characteristics. In addition, as a dairy product, it has a favorable image because of a clean label. WPC should be free of bixin or β -carotene colorant generally used in Cheddar cheese manufacture. To remove the colorant, the whey is bleached with hydrogen or benzoyl peroxide during the WPC process. Cheese plants manufacturing Swiss and mozzarella cheese use no colorants.

In general, whey proteins of WPC lack opacity and white appearance as compared to caseins present in NFDM. In general, WPC (with 34% protein level) concentration in pudding mix may range from 0.5 to 1% level and the rest of the milk solids are derived from NFDM.

Milk Protein Concentrate

Milk protein concentrate obtained by ultra-filtration of skim milk is a functional ingredient to raise protein level of the mix, but the main reason for its use is to

reduce lactose content of the mix to produce low-carbohydrate/lactose product. The lactose level can be significantly reduced as much as 70% by judicious use of lactose-reduced milk protein concentrate and high-protein WPC in the formulation and replacing milk and NFDM.

Since pudding is a manufactured product, its chemical composition is likely to vary depending on the quality standards established by marketing considerations. In any case, it is extremely important to standardize and control the day-to-day product in order to meet consumer expectations and regulatory obligations associated with a certain brand or label. The mix is formulated to predetermined milk fat and milk solids-not-fat content and the weights of each ingredient are calculated with the aid of computer software. Most manufacturing plants are equipped with computer programs to calculate the amounts of each ingredient needed to achieve target levels of milk fat, milk solids-not-fat, total solids, sugar, stabilizers, and other ingredients. The program usually also calculates cost of the mix.

Pudding may be manufactured from the whole milk (3.25% fat). Low-fat pudding is manufactured from mix containing 0.5–2.0% milk fat before the addition of bulky flavors. Nonfat pudding mix has milk fat level not exceeding 0.5%. These fat levels correspond to the FDA requirement for nutritional labeling (Chandan 1997). In some puddings available in the market, milk fat is replaced with vegetable fat.

SWEETENERS

Nutritive Sweeteners

Sugar. In the manufacture of pudding, a sweetening agent is added to the pudding base. The level of sweetness in the mix will depend on the desired level of sweetness in the finished product. Typical puddings contain approximately 13–16% sugar equivalent. The sweetener most commonly used in the industry is sucrose in either liquid (65–67% total solids) or granulated form. When liquid sugar is used, the added water is taken into consideration to avoid dilution of the total solids of the mix. The addition of the sugar generally occurs before pasteurization due to the following reasons:

- Heat treatment of the milk destroys any osmophilic yeasts and molds that might be present in the sugar ingredient.

- Potential source of post-pasteurization contamination (HACCP).
- The consistency of pudding is better when sugar is added to the milk rather than into the set base.

If it is necessary to add sweeteners after starch setting, only pasteurized liquid sugar or flavored sweetened syrups should be used. When using this method, the total solids of the mix must be adjusted for the dilution associated with these liquid sweeteners. Also, good manufacturing practices and HACCP control should be practiced to minimize the potential risk of microbiological or physical contamination.

Refined crystalline sucrose is manufactured industrially from sugar cane or sugar beet processing (Alexander, 1997). Both sources give identical sucrose with no chemical, physical, or structural differences. Crystalline sugar is either refined from crude raw sugar or is processed from sugar cane juice. The first step is to express juice from sugar cane using a series of roller presses. Nonsugar impurities are removed by mechanical filtration followed by lime-carbon dioxide purification step. The juice is allowed to settle and then filtered to get purified juice. In some factories, this step involves lime-phosphoric acid floatation procedure. Further purification of the juice is achieved by treatment with activated charcoal and ion exchange reactors. This juice (12–15% total solids, 91–92% purity) is evaporated in multistage vacuum evaporators to get sugar concentrate containing 65–71% solids. Further, crystallization of sugar is effected in vacuum pans under controlled conditions of temperature, pressure, density, and viscosity. The resulting sugar crystals are separated from mother liquor by centrifugation at 1,000–2,500g. The semidry sugar is rinsed with water and dried further with hot air in a rotating drum, cooled, classified on vibrating screens, and packaged. The mother liquor goes through a series of crystallization steps to harvest maximum yield of premium quality sugar. The left over liquor is a by-product of sugar industry, called blackstrap molasses.

Refined cane sugar is also manufactured from raw sugar produced at the point of origin. In this case, raw sugar is refined by extracting cane sugar juice, clarification, concentration, and crystallization. Other products from raw sugar production are white sugar, turbinado sugar, and various grades of molasses. Raw sugar is then shipped to sugar refinery where it is subjected to a series of purification steps, such as centrifugation, filtration, decolorization, evapora-

tion, and crystallization. The by-products of refining steps are brown sugar, refiner syrups, liquid sugar, and molasses.

Beet sugar is produced in a single step. Beets are sliced, followed by diffusion of sugar in water, clarification, concentration, and crystallization directly to white sugar.

High-purity sugar is 99.90–99.95% sucrose. When granulated sugar (high purity) is used for pudding production, it is purchased in 50–100 lb. bags, 1,000–2,000 lb. tote bags, or in bulk. In large plants, bulk sugar is stored in silos. The color of sugar is measured by procedures approved by International Commission for Uniform Methods of Sugar Analysis. The procedure involves measuring absorbance of 50% sugar solution (filtered through 0.45 micron membrane filter at 420 nm wavelength). The absorbance is converted to International Color Units (ICU). The higher the ICU number, the darker is the sugar color. Generally, most granulated sugars fall below 35 ICU. The inorganic ash content of sugar is approximately 0.02%.

The moisture level in sugar is less than 0.04%. Part of the moisture in sugar results from the syrup trapped within the crystal during its formation, which can be removed only by grinding sugar crystals. Another type of moisture is bound water associated with saturated syrup enveloping the crystals. Free moisture is attributed to a supersaturated solution coating the sugar crystal during rapid drying process of sugar manufacture. Further crystallization of the supersaturated solution during storage of sugar causes the free water to be released in the surrounding air. The dried granulated sugar is conditioned by the manufacturer to reach equilibrium with the surrounding atmosphere.

The size of crystals is selected for quick dissolution during the mix preparation. The crystal size distribution is normally defined by the percent of the crystals retained on standard U.S. mesh screen. The higher the mesh number, the finer would be the crystal size. Regular fine and extra-fine grade of sugar has fine crystals. The grain size ranges from U.S. #20/40 and #100 mesh screens. It is preferred by dairy processors for its bulk handling properties and resistance to caking or lumping during storage.

The rating for sweetness varies according to the crystalline form and size. It is related to the stereochemistry of the structural units in the sugar.

Liquid Sugar. Many large plants prefer using liquid sugar because it lends itself to efficient handling

(metering and pumping ability). Although liquid sugar may be economically priced, conversion from dry sugar to liquid sugar set-up requires capital cost for sugar storage tanks, appropriate pumps, heaters, strainers, and meters. The storage space and inventory control of liquid sugar must be coordinated with plant production volumes. If the delivery of liquid sugar is by tank cars, storage capacity requirements are of the order of at least 1.5 cars or 12,000 gallons. If truck delivery is convenient, the volume may be in the range of 1,000–3,000 gallons per delivery. To cope with emergencies like delays and increased usage, the inventory should be adjusted accordingly.

Liquid sugar is obtained by dissolving refined granulated sugar in water. Some cane sugar refining plants produce liquid sugar directly prior to crystallization and drying. It is delivered in tanks and stored in pudding plant in specific tanks equipped with ultraviolet light to control growth of yeasts and molds. Adequate ventilation of the tanks is necessary to avoid moisture condensation and resulting microbial growth. Storage temperature range is 30–32°C. This ingredient contains 66–67% solids (67°Brix) consisting of minimum of 99.7% sucrose and invert sugar level <0.35%. The ash content is restricted to less than 0.04% and iron content may not exceed 0.5 ppm. The pH is within the range of 6.7–8.5. A gallon of liquid sugar has 7.42–7.55 lb. of solids and weighs 11.08–11.12 lb. The viscosity of liquid sugar is around 2 poises. The color of liquid sugar is similar to that of granulated sugar (less than 35 ICU).

Conversion of mix formula from dry sugar to liquid sugar can be done as follows:

$$\begin{aligned} &\text{Pounds of liquid sugar required} \\ &= \frac{\text{Pounds of dry sugar required}}{\text{Percentage of solids in liquid sugar}} \end{aligned}$$

Normally, for 100 lb. of dry sugar, 149.25 lb. of liquid sugar is needed to add the same amount of sucrose in the formula.

More often, conversion of dry sugar to gallons of liquid sugar is required. To calculate gallons of liquid sugar to replace dry sugar, divide the pounds of dry sugar with pounds of sugar solids per gallon. To replace 100 lb. of dry sugar, gallons of liquid sugar required would be: $100/7.42 = 13.48$ gallons of liquid sugar.

Corn Syrups. Corn syrups made by hydrolyzing cornstarch are rarely used in pudding manufacture.

They are more commonly used in frozen custard manufacture. Corn syrups are defined as the products in which 20–70% of the glucoside linkages have been hydrolyzed. Three types of corn sweeteners are common in frozen yogurt industry. They are classified as low conversion (28–38 DE), regular conversion (38–48 DE), intermediate conversion (48–58 DE), and high conversion (58–68 DE). High conversion syrups may be obtained by a combination of acid and enzyme action on starch. High maltose syrup is made from a combination of acid and β -amylase hydrolysis. The disaccharide maltose consists of two molecules of glucose. Dry corn syrups are obtained by spray drying partially hydrolyzed cornstarch of various DE. Crystalline dry forms of refined dextrose and fructose are available. Generally, frozen pudding/custard producers use 36 or 42 DE corn syrup in liquid form or as dry corn syrup solids. Since the liquid form is very viscous, to facilitate their pumping and metering, this ingredient is stored in heated tanks at 32°C.

Corn syrup solids contribute firmness and extend shelf life of the frozen dessert. The high-polymer content contributes adhesive and cohesive properties to mix (Marshall et al., 2003). The corn syrup solids ingredient is a white powder and is susceptible to caking when exposed to moist air. Since too much corn syrup in the mix may impart a flavor defect, its use in frozen dessert is limited to one-third of the total sweetener level. Crystalline dextrose is a white powder with 80% of the sweetening power of sucrose. Dextrose, being a monosaccharide of molecular weight nearly one-half of sucrose, depresses the freezing point of the mix twice as much as sucrose. Frozen custard from a mix containing corn syrup displays less stiff consistency as it extrudes from the ice cream freezer. Accordingly, its usage level is adjusted not to exceed 25% of the total sweetener level.

High fructose corn syrups (HFCS) and crystalline fructose equal or exceed the sweetness of sucrose. HFCS production involves dextrose conversion to fructose in corn syrup by enzymatic means. They also lower the freezing point of frozen dessert mixes to the same extent as the original corn syrups.

Nonnutritive High Intensity Sweeteners

Puddings made with nonnutritive sweeteners are labeled as “no sugar added.” They are attractive to consumers interested in reducing intake of carbohydrates or calories in their diet. While replacing sugar with high-intensity sweeteners, it is necessary to

Table 17.2. High-Intensity Sweeteners Approved by FDA for Use in Certain Foods

Nonnutritive Sweetener	Sweetness Factor, Sucrose = 1
Aspartame	160–220
Sucralose	600
Acesulfame K	200
Neotame	7,000–13,000

incorporate bulk agents like maltodextrins, polydextrose, and so forth.

The following nonnutritive sweeteners are approved by the FDA for use in foods (Table 17.2).

Aspartame. Aspartame is a dipeptide. It is L- α -aspartyl-L-phenyl alanine methyl ester. Intestinal esterases hydrolyze to individual peptides and methanol. The end products do have calories, but since the level used is so small, the calorie contribution is essentially zero. However, aspartame breaks down by heat treatment in the manufacture of pudding. In addition, use of aspartame requires the statement “Phenylketonurics: contains phenyl alanine.” Accordingly, aspartame is generally not used in pudding manufacture.

Sucralose. Sucralose is another high-intensity sweetener which is truly nonnutritive. It is the preferred nonnutritive sweetener in no sugar-added pudding. It is poorly absorbed in the gastrointestinal tract (11–27%). The absorbed sucralose is excreted intact in the urine; the unabsorbed portion is excreted in the feces. This is how it provides no calories. It is synthesized from sucrose by replacing three hydroxyl groups with chlorine. Chemically speaking, it is 1,6-dichloro-1,6-dideoxy- β -D-fructofuranosyl-4-chloro-4-deoxy- α -D-galactopyranoside. It is three times sweeter than aspartame or 600 times sweeter than sucrose. It is stable to heat and acidic conditions prevalent in food processing and storage.

Acesulfame-K. Acesulfame-K is 5,6-dimethyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide. In general, the potassium salt is used. It provides no calories because 95% or more is excreted unchanged in the urine. It is 200 times sweeter than sucrose. It is stable to baking and cooking temperature. It works well with other nonnutritive sweeteners by providing sweetness synergy and masking unpleasant flavors.

Neotame™. Neotame™ was approved by the FDA on July 5, 2002. It is 7,000–13,000 times sweeter than sucrose. Like aspartame, it is a derivative of dipeptide of aspartic acid and phenylalanine. Since it is rapidly metabolized by esterases and the end products are excreted in body wastes, it is noncaloric. Compared to aspartame, phenylalanine released in plasma is not significant. Therefore, neotame™ requires no warning label for PKU. Its flavor is clean and sweet with no off-flavors. It lacks metallic flavor and enhances other flavors. It is heat stable and can be incorporated directly in pudding products.

Typical usage level of aspartame, sucralose, and acesulfame potassium to achieve 12–15% sucrose level in pudding is of the order of 0.14%, 0.03–0.05%, and 0.03–0.05%, respectively. Current trend in the use of high-intensity sweeteners in foods and beverages is to blend two or more sweeteners to optimize sweet flavor profile. A combination of acesulfame-K with aspartame, or sucralose can enhance perceived sweetness, optimize flavor, reduce cost, and improve sweetness stability in certain products.

NATIVE AND MODIFIED STARCH

Puddings can be formulated and processed for refrigerated or ambient distribution and storage. The short shelf-life product is processed in conventional heat-treatment systems and packaged in nonaseptic conditions and must be refrigerated at all times. However, most pudding products in the market are the heat sterilized, followed by aseptically packaging and are marketed under ambient storage conditions.

For more discussion on starch, the reader is referred to DeMiller (2003). Originally, pudding and custards were produced from milk and cornstarch using batch-processing in vats. Cornstarch gave a typical consistency and texture to the pudding and a “starchy” eating quality. Modified waxy maize starch produces a smooth texture, imparts stability to the product even at low temperature, and provides temperature and shear resistance. Modified tapioca improves spoonability.

At present, commercial puddings are not made with native cornstarch because of stability issues. Native starch will not survive ultrahigh heat treatment. Generally, pudding made with native starches exhibits shrinkage with a tendency for a milky liquid to separate out after refrigerated storage for 1–2 weeks. By selecting modified starches available, both

shelf-stable and refrigerated puddings have a built-in freeze-thaw stability to allow consumers extended shelf life with frozen storage.

A new technology has been developed for producing native starches without chemical modification that have properties similar to modified starches for application in low to moderate temperature and shear food systems. This functional native starch can be derived from corn or tapioca. Although these specially processed native starches are designed to resemble the textural properties of modified starches, they have both product and process limitations. For most applications, starch products that have been subject to chemical and physical modification result in starch gels that are made to withstand processing conditions involving high heat, shear, and freeze-thaw conditions. Improvement in gelatinization and pasting characteristics, solubility, and clarity are possible with appropriate modification. Furthermore, modification of starch can lead to viscosity generation or reduction, freeze-thaw stability, increased gel strength and enhanced appearance, and syneresis control, making them versatile for use in pudding processing.

Modified starch is the basis of texture generation in pudding products. It is used at 3–6% level in pudding formulation. Lower levels would be employed to create a less gelled structure, whereas higher level would yield more firm gelled structure.

Modified starches are derived mainly from corn-starch, tapioca, and waxy maize starch. From pudding manufacture standpoint, there are two classes of modified starch. One class is cross-linked and stabilized starch, which is basically used for creating viscosity. Selection of appropriate modified starch to optimize swelling property and maximizing viscosity should be based on the pudding process and packaging conditions. The process of modification involves cross-linking and stabilization. Cross-linking relates to esterification of hydroxyl groups of starch with phosphorus oxychloride (POCl_3). In addition, cross-linking of starch chains with phosphate diester reduces the degree and rate of granule swelling, which helps stabilize the product and provide resistance to break down during mechanical shearing. Stabilization relates to etherification with acetic anhydride or propylene oxide.

The second class of starches encompasses converted starch obtained by treatment with acid, enzymes, or certain chemicals. Acid-converted starches derived by reaction with hydrochloric/sulfuric acid impart mouthfeel enhancing properties, whereas

enzyme-converted starches produce specialty maltodextrins with mouth-coating properties. Oxidizing agents, like potassium permanganate or hydrogen peroxide, also produce converted starches to manipulate mouthfeel characteristics. These starches create special mouth-coating attribute to enhance creamy texture in pudding. A combination of these treatments may be applied. It is important to select the type of starch according to the pudding process. For kettle or batch process, it is desirable to select less cross-linked starch. Starch with lower cross-linking would get overcooked at higher temperatures used in ultra-high temperature (UHT) processing, leading to lower viscosity. In general, highly cross-linked starch is optimum for use in UHT processing.

Modified starches for specific use in various types of puddings are available from starch manufacturers. These starches are modified by combinations of cross-linking and stabilization processes applied to corn, waxy maize, and tapioca starches to create desirable functional attributes. Such attributes are solubility, pasting characteristics, cook temperature, hydration rate, adhesion, and moisture retention capacity, viscosity at low to high temperature, sheen, mouthfeel, color, freeze-thaw stability, film-forming ability, and stability to exposure to heat, shear, and cold. Pudding manufacture uses either batch process (kettle cooking) or UHT process in scraped surface heat exchanger. Most refrigerated pudding in the industry is UHT-treated but is not aseptically packaged. This system gives a shelf life of approximately 6 weeks. However, pudding produced with UHT-treatment and packaged aseptically gives a shelf life of 12–18 months under refrigeration. Shelf-stable (nonrefrigerated storage) pudding has considerably less shelf life.

All modified starches are pre-gelatinized. For use in dry instant pudding mixes, pre-gelatinized cross-linked modified starch is subjected to cooking, followed by spray drying or drum drying. On contact with cold or ambient temperature water, an instant gel is formed. Starch upon proper cooking becomes soluble and loses birefringence property (Rapp, 1986). Chemically modified starches are described in the U.S. Code of Federal Regulations, Title 21, Section 172.892.

In the manufacture of rice or tapioca pudding, starch is generated in situ by the rupture of rice grains or tapioca during the cooking process.

Table 17.3 lists various starches used in food product manufacture.

Table 17.3. Starches Used in Various Foods

Starch	Amylose (%)	Amylopectin (%)	Gelatinization Temperature (°C)
Dent corn	26	74	62–73
High-amylose corn	50–70	30–50	63–92
Waxy corn	1	99	62–73
Oat	16–29	71–84	53–59
Wheat	25	75	52–63
Rice	17	83	69–78
Waxy rice	1	99	64–72
Potato	20	80	56–62
Tapioca	16	84	58–70
Sorghum	24	69	75
Waxy sorghum	1	99	71–76

Adapted from Rapaille and Vanhemelrijck (1998); Jackson (2003); and Ratanayake and Jackson (2003).

HYDROCOLLOIDS

They act as thickeners and stabilizers. The primary purpose of adding stabilizers is to improve consistency and build viscosity, to minimize whey separation and bind-free water, and to maintain the gel structure after pumping, mixing, and cooling. The stabilizer increases shelf life of the product and provides a reasonable degree of uniformity from batch to batch. Stabilizers function through their ability to form gel structures in water, thereby leaving less free water for syneresis. In addition, some stabilizers complex with casein, the major milk protein. A good stabilizer should not impart any flavor, should be effective at low concentration levels, and should be easily dispersed in the normal working temperatures in a pudding plant. In addition, the stabilizer should be easily soluble, display good water holding capacity, and aid in forming stable emulsion. Furthermore, it should promote gelation and adhesion.

The stabilizer system used in pudding preparations is generally a combination of starch and carrageenans. However, to build special properties, various vegetable stabilizers may be employed. Their ratios as well as the final concentration (generally 0.05–3.00%) in the product are carefully controlled to get desirable effects. For choosing a stabilizer, the following areas should be considered:

- Formulation: fat content, type of fat, milk fat or vegetable fat, total solids.
- Interactions with milk constituents for possible synergy or interference with the ingredients of pudding mix.
- Desired firmness and consistency of the finished product as per marketing objectives.

- Desired ingredient labeling (natural, organic, kosher, and halal).
- Processing equipment available: batch process (ease of incorporation), continuous heating system, in-line dosing and mixing, cooling, and pumping of coagulum.
- Possible masking effect on the vanilla and other flavors.

During processing, the incorporation of the stabilizer should take place using a Lanco- or Breddo-type liquefier that has strong agitation resulting in complete dispersion and a uniform suspension. An alternative method would be to use a pump and funnel, but care must be taken to avoid lumps. To minimize potential lumps or “fish eyes,” it is best to disperse the stabilizer in granulated sugar or NFDM during addition. Once dispersed in the mix, it is necessary to have continuous agitation to keep the stabilizer in suspension until it is fully hydrated while receiving proper heat treatment.

Carrageenans

In pudding manufacture, carrageenans are commonly used as a thickener and stabilizer. Carrageenans consist of mixtures of various galactans in which sulfate half esters are attached to sugar units. Their origin is red seaweed. To prepare carrageenan, the red seaweed is treated with potassium hydroxide solution to extract and remove soluble compounds. The insoluble potassium carrageenans are dried and ground to a flour. They are subsequently treated with sodium hydroxide solution to extract the soluble sodium salt of carrageenan. Carrageenans comprise kappa-, iota-, and lambda-carrageenan with

Table 17.4. Use of Various Carrageenans in Dairy Desserts

Type	Properties and Mode of Action	Gel Structure
Kappa	Sodium salt is soluble but potassium and calcium salts are insoluble in cold water or milk. However, all salts are soluble at temperature higher than 65°C in hot water or milk.	Low concentrations in milk generate stiff, brittle, and thermoreversible gel by interaction with potassium and calcium.
Iota	Sodium salt is soluble but potassium and calcium salts are insoluble in cold water or milk. However, all salts are soluble at temperature higher than 55°C in hot water or milk.	Low concentrations yield soft, resilient, and thermoreversible gel in milk. The gel does not synerese and is freeze-thaw resistant.
Lambda	All salts are soluble in cold and hot water or milk.	Thickens cold milk.

Adapted from BeMiller and Whistler (1996).

distinct functional characteristics. They complex with milk proteins to form different types of gels. Table 17.4 summarizes the use of carrageenans in pudding manufacture. Kappa-carrageenan is generally used at 0.10–0.15% and iota-carrageenan concentration varies from 0.09 to 0.11%. Choice of carrageenan is determined mainly by whether the product is cold-filled or hot-filled. Several American pudding manufacturers hot-fill their pudding products. Kappa-carrageenan gives a brittle, thick gel, while iota-carrageenan produces soft gel which on cold-filling gives very slick and smooth texture. A combination with modified starch imparts more creamy impression in the mouth. Therefore, a combination of carrageenan and modified starch is widely spread practice in the pudding industry to achieve an optimum final texture.

In flan-type products, carrageenan facilitates gel formation and assists in unmolding of the product. In creamy products, it assists in desirable consistency and thixotropic behavior.

Other Stabilizers

Algin and sodium alginate are derived from giant sea kelp. These stabilizers are heat stable and promote stabilization of the gel by complex formation with Ca^{+2} and casein. Pectins are occasionally used alone or in combination with other hydrocolloids to stabilize the structure of pudding. Very small amounts (0.07–0.15%) modify the consistency of the milk gel making it stiffer and preventing any syneresis that might arise during handling, transportation, and dis-

tribution. Low-methoxy pectin retains the whey in a very flexible network that is formed in reaction with calcium ions present in the pudding. The maximum amount of pectin to be added is 0.20%, as higher concentrations could result in a chalky or sandy texture and decreased viscosity.

Among the seed gums, locust bean gum or carob gum is derived from the seeds of a leguminous tree. Carob gum is a neutral polysaccharide and therefore pH has little effect on viscosity in the range pH 3–11. It is insoluble in cold water and must be heated to be dissolved. It does not have gelling properties on its own and in pudding it may be used primarily to add viscosity or increase gel strength in combination with other stabilizers. Its principle function is stabilizing and the binding of water.

Guar gum is also obtained from seeds and can be used in stabilizer systems for refrigerated or frozen pudding. Guar gum is readily soluble in cold water and is not affected by high temperatures used in the pasteurization of sterilized pudding. Guar gum is nongelling and is used mainly as a viscosity builder, stabilizer, and moisture-binding agent.

In some formulations, certain calcium interacting gums produced by fermentation processes are used. For example, gellan gum is a linear extracellular, anionic polysaccharide secreted by the microorganism *Aurumouas elodea*. Gellan gum is sensitive to calcium ions of milk thereby forming gels similar to alginates. They exert thickening effect and bind water thereby stabilizing body of the pudding and may replace alginates in pudding formulation. The usage level is 0.05–1.5%. The texture achieved is not quite identical to that obtained with starches.

Carboxy-methyl cellulose is a derivative of the natural product cellulose. It is readily soluble in either hot or cold water and is effective at high processing temperatures. Its primary function would be as a thickener and moisture-binding agent. By judicious combination of various hydrocolloids, it is possible to produce moldable pudding that retains its shape after removal from the cup.

POLYPHOSPHATES

They are used to control the degree of protein aggregation induced by heat treatment of exceeding 129.4°C necessary for sterilizing the pudding mix. The protein aggregation is more noticeable in low to nonfat pudding and is characterized by white speckled and translucent appearance accompanied by chalky mouthfeel. These attributes are considered defects in the product. The protein aggregates are of the order of 40 μm in size. The phosphate mixture comprises equal weights of tetra sodium pyrophosphate and disodium dihydrogen pyrophosphate (also called sodium acid pyrophosphate) at a level of 0.05–0.5% by weight of pudding (Leshik, 1993).

EMULSIFIERS

Some pudding products are formulated to replace milk fat with vegetable oil or fat. To help emulsify them, certain emulsifier are used. Nevertheless, in all puddings, the emulsifier aids in the dispersion and mixing of dry ingredients and formation of relatively firm and smooth texture. Emulsifier help in achieving texture and aeration. The emulsifier consist of mono- and diglycerides prepared by direct esterification of edible fatty acids and glycerin. Other emulsifier are acetylated monoglycerides, propylene glycol monoesters, and glycerol lacto palmittates. A commonly employed emulsifier is sodium stearoyl-2-lactylate. Most emulsifier are used at low levels (0.02–0.08%). Its level is raised to 0.15% when milk fat is replaced with vegetable fat.

SALT AND EGG (WHITE AND YOLK)

These are commonly used in pudding formulation. Salt is used to round off the overall flavor and is generally used at 0.20–0.30%. Egg yolk (frozen, sugared) imparts a characteristic flavor as well as a source of lecithin emulsifier. Liquid pasteurized whole egg is used in certain rice puddings.

COCOA

Cocoa is produced from partially fermented fatty seed of the cocoa tree. It is a powder obtained by grinding cocoa solids following the removal of cocoa butter. Cocoa beans are roasted to get cocoa nibs which are finely ground to obtain chocolate liquor. The cocoa mass is then pressed to fractionate it into cocoa cake and cocoa butter. The cocoa cake is then ground to get cocoa powder (Biehl and Ziegler, 2002). Natural cocoa powder is obtained from cocoa beans without treatment with alkali. It is lighter in color and has a harsh bitter taste, whereas Dutch-processed cocoa is darker in color and has a milder smooth flavor. Dutch process refers to treatment of cocoa nibs with alkali. Cocoa powder may contain varying levels of fat. Cocoa powder contains fat content of 17–30% and chocolate liquor contains 50–55% fat. In general, cocoa powder of 22–24% fat at 1.5–2.5% level is used in pudding manufacture. In addition, chocolate liquor at 1.5–2.0% aids in improvement of chocolate flavor.

VANILLA

This flavor is a very popular flavor of commercial pudding. Most of the vanilla beans (65–70%) come from Bourbon islands (Madagascar, Comoro, Reunion, and the Seychelles). Indonesia and India supply 25–30% of the world's bean production. However, Bourbon beans are considered as the source of fine vanilla. Vanilla beans are derived from the fruit of *Vanilla fragrans*. This plant belongs to orchid family. The beans are harvested and cured. During this process, fermentation and "sweating" of beans gives rise to methyl vanillin, the predominant flavor of natural vanilla extract. To prepare the extract, beans are extracted with a mixture of water and alcohol. Optional ingredients of extracting solvent are glycerin and sugar. One gallon of standard strength vanilla extract is equivalent to 13.34 oz. of vanilla beans. Alcohol content of the extract ranges from 30 to 50%. By evaporating solvent, concentrated extracts (2–5-fold) are also available (Marshall et al., 2003). Some processors prefer powdered vanilla because it does not cause dilution of pudding with vanilla solvent, alcohol. To prepare the powder, vanilla beans are ground with sugar. Specks of vanilla beans are visible in this type of powder. If no specks are required, the powder is obtained by drying under vacuum a blended paste of single strength vanilla extract and sugar. The proportion of vanilla

Table 17.5. Typical Composition of Refrigerated Milk-Based Pudding

Component	Vanilla (%)	Light Chocolate (%)	Dark Chocolate (%)
Milk fat	3.50	3.50	3.5
Milk solids-not-fat	8.25	7.50	7.50
Sucrose	14.75	16.00	16.00
Modified starch	5.80	5.70	5.10
Vanilla	As needed	As needed	As needed
Color	As needed	—	—
Cocoa	—	1.40	2.50
Total solids	33.30	34.10	34.60

Adapted from Chandan (1997).

extract and sugar is designed to yield single strength vanilla powder.

Artificial vanilla flavor is prepared from synthetic methyl vanillin. This flavoring offers cost savings because of its flavor potency but the label of the product must indicate artificial flavor. Furthermore, its flavor balance and aroma are considered less desirable than those of natural vanilla. In relation to flavor strength, 0.7% solution of vanillin is equivalent to 1 lb. of dry vanilla beans. Pure vanilla flavoring has a standard of identity (FDA 21 CFR 169.175). Mixtures of pure vanilla and vanillin are covered in FDA 21 CFR 169.177. Imitation vanilla is identified in FDA 21 CFR 169.181.

In pudding manufacture, vanilla (2×) is generally used at a level of 0.25–0.30% in vanilla pudding and at a level of 0.10–0.15% in chocolate pudding.

BUTTERSCOTCH

Another popular flavor associated with puddings is butterscotch. It is made by boiling a mixture of sugar syrup, butter, cream, and vanilla. For application in pudding manufacture, this flavor may be procured

as liquid butterscotch topping or as dry butterscotch chips. Butterscotch topping is generally used at 9–14% level.

GENERAL PROCESSING PROCEDURES FOR MAJOR TYPES OF PUDDING

REFRIGERATED READY-TO-EAT PUDDING USING STARCHES

Tables 17.5 and 17.6 show formulation of typical refrigerated puddings.

Kettle/Batch Process (Hot Pack)

Nonsterile dairy puddings may be manufactured by the hot-pack procedure exemplified below. Milk fat in the pudding can be varied using milk or skim milk blends. Vegetable fat can be substituted for milk fat, if desired.

A general manufacturing procedure for refrigerated milk-based pudding is briefly described below.

Table 17.6. Formulation for 1000-lb. Batch of Refrigerated Dairy Pudding

Ingredient	Vanilla (lb.)	Light Chocolate (lb.)	Dark Chocolate (lb.)
Whole milk	750	732	726
Cream, 40% fat	25	25	25
NFDM, Low heat	18	13	13
Sucrose	148	160	160
Modified starches	58	57	51
Flavor and color	As needed	0	0
Cocoa	0	14	25

NFDM, nonfat dry milk.

Adapted from Chandan (1997).

Table 17.7. Suggested Formulation for Long-Life, Aseptically Packaged Vanilla, Chocolate, and Butterscotch Pudding

Component	Vanilla (%)	Chocolate (%)	Butterscotch (%)
Milk, 3.3% fat	63–68	56–63	63–68
Sugar, granulated	13–16	12–17	7–10
Cream, 36% fat	7–8	3.5–4.5	3–6
Water	5.5–7.0	10–14	2.5–5.0
Cocoa powder	0	1.5–2.5	0
Chocolate liquor	0	1.5–2.0	0
Butterscotch topping	0	0	9–14
Cross-linked waxy maize starch	3.5–4.5	3.5–4.5	3.5–4.5
Vanilla extract, 2×	0.25–0.30	0.10–0.15	0
Salt	0.15–0.25	0.20–0.30	0.25–0.35
Kappa-carrageenan	0.10–0.20	0.10–0.15	0.25–0.35
Iota-carrageenan	0.07–0.14	0.09–0.11	0
Sodium stearyl-2-lactylate	0.03–0.05	trace	0.03–0.05
Color	As needed	0	0.02–0.04
Butterscotch fl vor	0	0	0.03–0.05
Calcium oxide	0	0	0.015–0.025

Adapted from Rapp (1986).

- Blend all ingredients by adding dry ingredients to the mixture of milk and cream in the mix kettle. Use powder horn and a suitable pump for the circulation and the efficiency of dispersion. Boil color solution. Add color and fl vor during heating to 65.6°C.
- Pump the mix to the processor for pasteurization. Heat to no more than 68.3°C in the processor and hold for 30 minutes for meeting pasteurization requirements.
- Pump the pasteurized product to the pudding thermutator or scraped surface heat exchanger (SSHE). A converted ice cream freezer (without the use of cooling system) has also been used by connecting steam supply in the jacket. Raise the product temperature to 90.6–93.3°C with steam pressure.
- Run the product from the SSHE to the hopper of the packaging assembly via holding tube so that packaged product in the cup is at 73.9°C. Apply lids on the cups.
- Invert the pudding cups in the wire cases. Allow adequate interspaces for proper cooling. Hold at room temperature for 10–15 minutes for pasteurization of the interior of the cup and lid before cooling.
- Cool to 10°C by transfer into freezer at –22°C.
- Make sure that the product is fanned extensively for the temperature to drop to 10°C in approximately 1 hour.

- After the product is at 10°C, transfer the wire cases to the cooler at <7°C and store overnight before putting it in shipping cases.
- Run fl vor, texture, and body tests. Analyze for starch granule size, fat, total solids, sugar, SPC, yeast and mold, coliform, and psychrotrophic counts. Release the product for shipment if it meets the standards.

Microbiological and other Standards

- Standard plate count—<100 CFU/g
- Coliform count—0
- Psychrotrophic count—0
- Mold and yeast count—0

Expiration Period

45–60 days at 5–7°C.

Sterilized and Aseptically Packaged Starch-Based Milk Pudding

A suggested formula is given in Table 17.7.

Figure 17.2 shows general steps for the manufacture of shelf-stable and extended shelf life (ESL) refrigerated pudding.

The sterilized pudding process requires more rigorous heat-treatment (Chambers and Nelson, 1993). Direct heating systems use live steam injection or infusion into the product. The UHT processing is normally conducted in plate heat exchanger, tubular heat

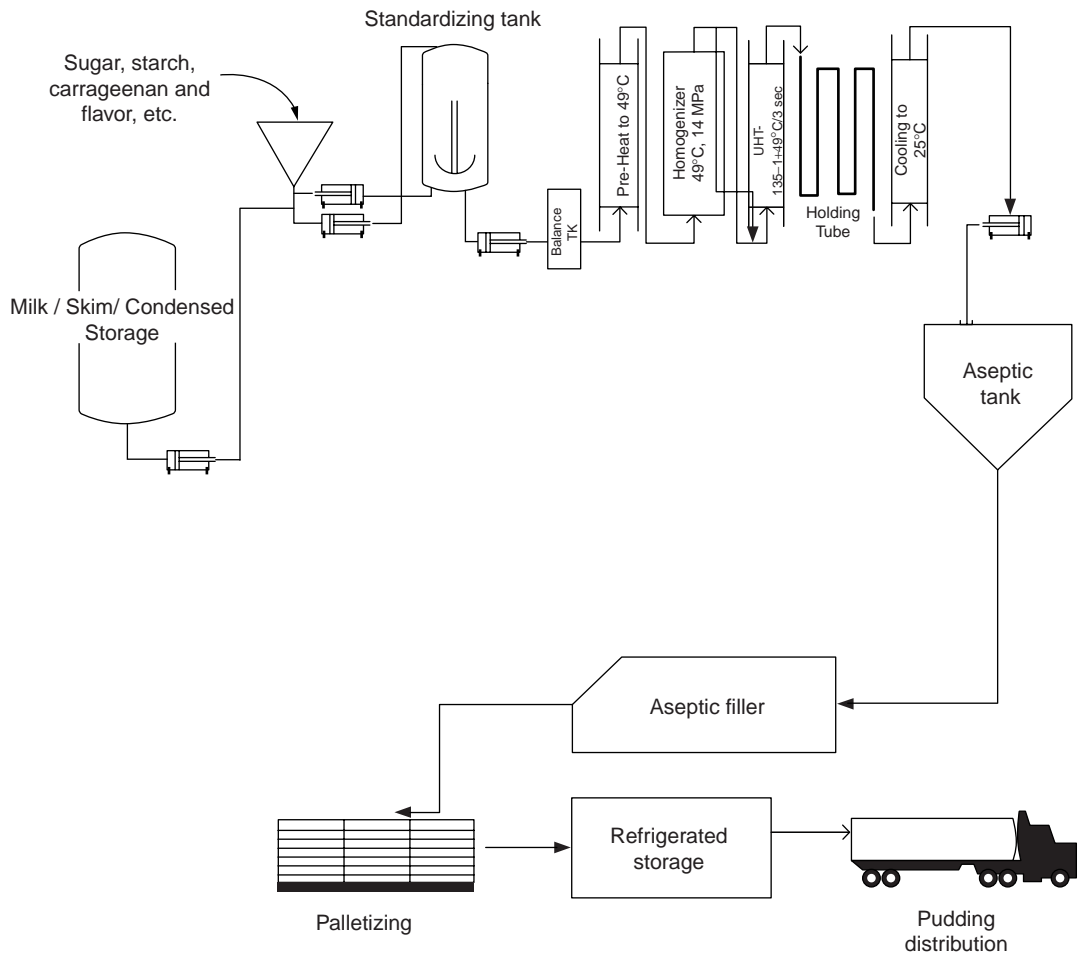


Figure 17.2. General manufacturing outline for smooth pudding.

exchanger, or scraped surface heat exchanger equipment. Such equipment is necessary to accommodate increases in viscosity during heat treatment of the mix.

The indirect heating systems are as follows:

1. Plate heat exchanger consists of horizontally or vertically arranged thin corrugated stainless steel plates. The product to be heated flows on one side and the heating or cooling medium flows in opposite direction in the other plate. Gaskets on the plates prevent intermixing of product and heating medium by isolating them from each other.
2. Tubular heat exchanger is a sterilizing system in which stainless steel coils for heat exchange. The diameter of the coil is designed to accommodate viscosity and flow characteristics. Among various designs, concentric triple tube is used for viscous fluids. The product is pumped through the middle tube and heating/cooling medium is pumped in the inner tube and outer tube. The product flow is opposite to the heating/cooling medium flow.
3. Scraped surface heat exchanger consists of a tube or barrel in which a central shaft is mounted with a series of blades. As the shaft goes into motion, the inner surface of the barrel is scraped constantly to prevent any buildup and burn on of the product. The barrel is equipped with a concentric tube (jacket) in which heating/cooling medium is circulated. The processed product is cooled and aseptically packaged.

It is common to subject the mix to preheating temperature range of approximately 49°C. In subsequent step, the preheated mix enters the UHT system where the temperature is raised to 135–148.9°C. At this point, the mix enters a holding tube where the mix is held for appropriate time (2–3 seconds) to insure cooking of the pudding and effective microbial kill to achieve sterility of the product. Thereafter, the cooked pudding is cooled to a temperature range of 26–30°C and stored in aseptic vessel followed by packaging in aseptic environment. Pudding may be processed without the use of scraped surface heat exchanger, if the viscosity stays relatively low after aseptic heat treatment (Joseph et al., 1988). Such product is formulated by using 73.2% skim milk, 17.43% sucrose, low starch level (2.8% modified waxy maize starch), 5.62% vegetable fat containing hydrogenated coconut and palm kernel oils, melting point 38.9°C, 0.4% vanilla extract flavor, 0.2% salt, 0.15% color/flavor, and 0.2% sodium stearoyl 2-lactylate to develop low on-line viscosity after cooking and sterilizing stage. This product develops optimum viscosity only after refrigeration of packaged cups.

Single-serve containers are popular for packaging operation in a pudding plant. The pudding is packaged in form-fill system in which plastic containers are thermoformed or molded from packaging materials such as high polystyrene, filled with sterile pudding. Subsequently, the cups are sealed with lids cut from flexible lid stock. The lid material may be foil-laminated polyester with a heat-sealable coating which on application of heat makes a good seal of the lid to the container. All these steps are carried out in an enclosed aseptic system. If preformed cups and lids are used, they are unscrubbed and sterilized with one of the methods using superheated steam, hydrogen peroxide, ultraviolet light, or high-intensity light. Even flexible lid stock needs to be sterilized. If pudding temperature is not low enough at packaging stage, the sealed containers run the risk of deformation after cooling to refrigeration temperatures. In some instances, the seal may break as well. Therefore, it is imperative to package the product at 26–30°C. Also, the viscosity of the product should be optimum to avoid splash pudding on the cup which would cause seal problems due to poor adhesion of the lid to the cup.

The product filling temperature characterizes a refrigerated dairy dessert. Hot-filled desserts such as flavor are packaged hot into cups at temperature above the gelation point of the gelling agent (for example,

carrageenan) used. As the product cools, it forms the texture of a firm gel. On the contrary, to get minimum change in post-packaging texture, the processed product is cooled under sheer conditions as the gelling agent is setting up gel network. Cold-filling gives a creamy texture without the appearance of a gel. Puddings and mousses are, therefore, packaged at <15°C.

In some cases, some manufacturers do not have rigorous aseptic conditions for producing the truly aseptic product suitable for ambient storage of the pudding product. However, they can package sterilized pudding in clean containers under clean environment to produce ESL-refrigerated pudding.

A general processing procedure for aseptically processed dairy pudding is given below.

- Dry blend sugar, NFDM, corn syrup solids, guar gum or other stabilizers, carrageenan, salt, and modified starches.
- Add blend slowly with agitation to water or liquid milk at 26.7°C.
- Heat the mixture to 48.9°C.
- Add mixture of vegetable oil (if used) and emulsifier at 48.9°C to the aqueous blend. Add color and flavor. Mix thoroughly.
- Homogenize mixture at 48.9°C.
- Cook at 121–148.9°C through sterilization equipment and hold for 2–3 seconds, as recommended by the equipment manufacturer.
- Cool to 26.7°C, and hold in aseptic storage.
- Pack in aseptic packaging systems in form and fill plastic individual serving containers at ambient temperature.
- Run aseptic quality tests prior to release of the product for distribution.

VEGETABLE FAT PUDDING

Table 17.8 shows formulation for pudding in which milk fat has been replaced with an appropriate vegetable fat.

It is produced by a process similar to other aseptically packaged products. The vegetable fat is emulsified by homogenization in the presence of a functional emulsifier.

LOW- AND NONFAT PUDDINGS

Such pudding products are on the market to offer consumers choices for nonfat or low-fat versions. In the absence of fat in the mix, calcium-sensitive,

Table 17.8. Typical Formulation of Aseptically Packaged Vegetable Fat Pudding

Component	Vanilla (%)	Chocolate (%)
Sucrose	14.77	14.77
NFDM, low heat	5.18	5.18
Corn syrup solids, 36 DE	2.46	2.46
Guar gum	0.12	0.12
Carrageenan	0.05	0.05
Cocoa	0	2.92
Salt	0.09	0.09
Modified starch	3.12	3.00
Emulsifier	0.13	0.13
Vegetable fat	6.61	6.60
Water	67.47	64.68
Color	As needed	—
Flavor	As needed	—

NFDM, nonfat dry milk

Adapted from Chandan (1997).

irreversible gelling hydrocolloid, namely, sodium alginate, is a key ingredient to form desirable texture in UHT-processed pudding. Low-methoxy pectin and gellan gum are also effective in this regard (King and Leshik, 1993).

Table 17.9 gives the formulation of nonfat and low-fat pudding.

Processing procedure is similar to other sterilized aseptically packaged products.

NO SUGAR-ADDED PUDDING

The formulation of such pudding is exemplified in Table 17.10.

Table 17.10. Formulation of No Sugar-Added Pudding

Ingredient	Weight (%)
Milk, 2% fat	62.63
Modified starch	4.30
Flavor	0.21
Sodium stearoyl lactylate	0.20
Cocoa	2.80
Water	29.26
Lactic acid	0.31
Aspartame	0.14
Xanthan gum	0.15

Adapted from Leshik et al. (1990).

This formulation uses lower milk solids to obtain stability at pH of 5.7 and xanthan gum functions as a viscosity/body-building agent. The key to formulating this type of pudding is the use of high-intensity sweeteners, namely, aspartame, sucralose, and acesulfame-K. It is common to use a mixture of the high-intensity sweeteners to impart a better sweetness profile resembling sucrose. Aspartame is relatively heat labile while the other sweeteners used are heat stable. In case of aspartame, it has been found that it retains its stability during storage of pudding of pH 5.5–5.7. The process involves two-stream steps. In the first step, an aqueous solution of aspartame containing all the lactic acid (Table 17.10) is filtered, sterilized and stored aseptically. The second stream consists of the rest of the formulation ingredients which are blended and homogenized at 57°C in a two-stage homogenizer (2,000 psi at first stage and 500 psi at second stage). The mix is then sterilized

Table 17.9. Typical Formulation of Fat-Free and Low-Fat Pudding

Ingredient	Fat-Free Chocolate (%)	Fat-Free Vanilla (%)	Low-Fat Vanilla (%)
Milk, 2% fat	0	0	66.09
Skim milk	70.00	71.01	0
Water	9.88	10.55	18.10
Sucrose	12.79	12.28	10.10
Starch	4.41	5.00	4.10
Sodium stearoyl lactylate	0.20	0.20	0.20
Sodium alginate	0.15	0.10	0.23
Flavor and colors	0	0.70	0.36
Polyphosphates (50% tetrasodium and 50% sodium acid pyrophosphate)	0	0.08	0
Cocoa	2.57	0	0

Adapted from King and Leshik (1993) and Leshik (1993).

Table 17.11. Formulation for High-Protein, Low-Carbohydrate Pudding

Ingredient	Vanilla 1 (%)	Chocolate 1 (%)	Butterscotch (%)	Banana (%)
Water	84.7	83.78	80–90	80–90
Calcium/sodium caseinate	7.46	6.87	6–9	6–9
Soy protein isolate	5.56	5.56	4.5–7.5	4.5–7.5
Whey protein isolate	0	0	0–3	0–3
WPC	0	0	0–1.5	0–1.5
Soybean oil	0.88	0.58	0.75–1.75	0.75–1.75
Sodium chloride	0.44	0.44	0.1–0.5	0.1–0.5
Potassium chloride	0		0–0.4	0–0.4
Carrageenan	0.3	0.33	0.1–0.35	0.1–0.35
Dipotassium phosphate	0.36	0.12	0.3–0.45	0.3–0.45
Tricalcium phosphate	0.044	0.044	0.025–0.3	0.025–0.3
Sucralose	0.04	0.04	0.03–0.05	0.03–0.05
Acesulfame K	0.033	0.033	0.03–0.04	0.03–0.04
Vanilla fl vor	0.19	0	0	0
Cocoa	0	2.21	0	0
Butterscotch fl vor	0	0	0.1–0.05	0
Banana fl vor	0	0	0	0.1–0.5

WPC, whey protein concentrate.

Adapted from Scinto (2006).

by heat treatment of 130–150°C for 6–30 seconds (Leshik et al., 1990). The two sterile streams are then blended aseptically at 24°C. In this way, aspartame is not subjected to high temperatures required for sterilization. The sterilized pudding is then aseptically packaged. In the final pudding, lactic acid acidifies the pudding to reduce the pH from 6.6 to 5.6 and the pudding obtained does not exhibit acidic taste.

LOW-CARBOHYDRATE, HIGH-PROTEIN PUDDING

In this type of ready-to-eat pudding, no starch is used. Instead, its formulation includes proteins (sodium/calcium caseinate, soy protein isolate, and whey proteins), soybean oil, and other ingredients as shown in Table 17.11.

It is characterized by being high in protein, low in carbohydrate, and free of starch, hydrogenated fats, and trans fatty acids. The formulation is designed for refrigerated pudding processing as well as for aseptic shelf-stable processes.

LAYERED PUDDING FOR PARFAIT CONFIGURATION

The multilayered dessert may be decorated with a layer of whipped cream or fruit layer. After process-

ing of each layer of pudding, transparent cups are partially filled with layer-1 vanilla pudding, followed by layer-2 chocolate pudding, and layer-3 vanilla pudding on the top. Each layer should be distinctly visible with a clear-cut demarcation between the layers (see Table 17.12).

Aseptic pudding with puree has been described by Welch (2007).

FLAN-TYPE PUDDING

Flans are moldable gels which can be easily removed from their containers by placing the container upside down and punching a hole on the bottom to facilitate product removal. Flan may have a caramel sauce topping at the bottom of the container which flows from the top of the product after it is removed from the container. The texture of the dessert characterizes the product. The texture may vary from being firm with brittle, creamy, or cohesive mouthfeel. For egg custard, the use of eggs and baking procedure develops the desirable firm texture.

To prepare flan at home, sugar and thickening powders are blended and a small quantity of milk is added to form a paste. Milk is brought to boil and the paste is added with thorough mixing until a homogeneous mixture is obtained. It is then added to plastic cups with caramel sauce added to the cup beforehand.

Table 17.12. Typical Formulation of Parfait-Type (Layered) Pudding

Ingredient	Layer 1 Vanilla (Weight %)	Layer 2 Chocolate (Weight %)	Layer 3 Vanilla (Weight %)
Water	48.87	49.5	48.13
Condensed skim milk	22.64	22.0	24.03
Sugar	17.17	17.0	17.17
Hydrogenated cocoanut/palm kernel oil	5.62	4.6	5.62
Modified food starch	3.8	3.8	3.8
WPC, 35% protein	1.00	0	0
Phosphoric acid solution, 17.5%	0	0	0.32
Flavor	0.55	0.2	0.55
Sodium stearoyl lactylate	0.2	0.2	0.2
Salt	0.18	0.2	0.18
Cocoa powder	0	2.5	0

WPC, whey protein concentrate.

Adapted from Flango et al. (1990).

After cooling, the flan sets and is ready for demolding and serving (see Table 17.13).

The manufacturing procedure for flan is covered in a U.S. patent (Salmones, 2006).

The batch process comprises processing vat. Skim milk is pumped into the vat, followed by the addition of cinnamon, lemon rind, cream cheese, liquid eggs, and sugar. The mix is blended for 30 minutes. Liquid caramel (5–6 g) is dosed into 120 mL containers, followed by 100–105 g of the blended mix in each packaging cup. The containers are vacuum-sealed and heat treated at 70–105°C for 25–35 minutes. The set flan is cooled rapidly to 0–8°C. Flan without caramel topping or with other flavors can also be made. For coffee flavor, include 2–3% soluble coffee and for chocolate, add 10–12% dark chocolate coating.

A flan-type pudding has been developed which is egg-free and consequently is low cholesterol (Kadan

and Ziegler, 1990). Table 17.14 gives a suggested formula of such a flan.

The texture and body of traditional flan are formed as a result of denaturation of egg white. In the formulation given in Table 17.14, the desired texture is obtained by the use of rice starch, various hydrocolloids, and whey protein isolate. The proteins isolate functions similar to egg white. For processing and packaging, standard procedure used in aseptic pudding manufacture may be used.

AERATED DESSERT/MOUSSE

Mousse is an aerated and stabilized product light, dry, and foam-like consistency. Generally, mousse is prepared from heat-treated liquid mix which is

Table 17.13. Typical Formulation of Baked Flan

Ingredient	Usage Level
Skim milk	65 liters
Cream cheese	15 kg
Liquid eggs	20 kg
Sugar	14 kg
Flavor, cinnamon	125 g
Lemon rind	550 g
Liquid caramel	6 kg

Adapted from Salmones (2006).

Table 17.14. Formulation of Egg-Free Flan

Ingredient	Weight (%)
Water	81.670
NFDM	7.350
Sugar	8.160
Rice flour	2.000
Carrageenan	0.282
Locust bean gum	0.116
Pectin	0.065
Tetra potassium pyrophosphate	0.212
Xanthan gum	0.073
Whey protein isolate	0.073

WPC, nonfat dry milk.

Adapter from Kadan and Ziegler (1990).

Table 17.15. Suggested Formulation of Settable and Aerated Dessert

Ingredient	Chocolate	Lemon	Strawberry	Vanilla
Cream, 35% fat	16 liters	10 liters	10 liters	10 liters
Sugar	3 kg	2050 g	2.5 kg	2050 g
Gelatin	226 g	226 g	226 g	226 g
Cocoa, alkalized, 10–12% fat	600 g	0	0	0
Chopped chocolate	3 kg	0	0	0
Cream cheese	1200 g	1240 g	2 kg	1250 g
Lemon juice	0	2.7 liters	0	0
Condensed milk	0	4 kg	0	4 kg
Water	1200 mL	2 liters	2 liters	2 liters
Strawberries	0	0	1 kg	0
Vanilla	0	0	0	As needed

Adapted from Flynn (1999).

whipped in a continuous aerater, filled into cups, sealed, and cooled. Mousse can also be produced with an ice cream freezer, followed by packaging of frozen mousse in cups. The frozen mousse is marketed frozen and is thawed out prior to consumption at home by the consumer. The density of the product is controlled by the degree of whipping air and the resulting overrun. The overrun may be 100–150%, depending on the texture desired in the whipped product. It is important to use an effective emulsifier for the manufacture of mousse to create fine air bubbles. Gelatin is a key ingredient along with cream, cream cheese, and sugar. Flavorings such as fruits, cocoa, or vanilla may be used. Flynn (1999) has given the formulation (Table 17.15) and process of such a product.

To manufacture chocolate dessert, cream, sugar, cocoa, and chocolate chips are blended and beaten in a Hobart-type mixer to attain 25% overrun at 10°C. This premix is stored frozen or refrigerated for use in the final product. Five liters of premix is whipped, mixed with 1.5 gallons of cream and whipped further. It is then mixed with slurry consisting of 300 mL of water at 80°C, 300 g cream cheese, and 55 g of gelatin. The product is reported to have shelf life of 6 months in frozen storage and exhibits shelf life of 3 weeks in refrigerated conditions (Flynn, 1999).

To manufacture lemon mousse, cream cheese and sugar is mixed in Hobart mixer to a paste consistency. It is whipped to get 25% overrun at 10°C and the cream is blended and whipped further. At this point, lemon juice is added. Gelatin is dissolved in hot water and blended in. After mixing, the filling is ready for parfait rolls, cheesecakes, and Swiss rolls.

For strawberry mousse manufacture, sugar, cream cheese, and cream are blended to smooth consistency

and whipped to get 25% overrun at 10°C. At this point, strawberries are blended into the mix. The gelatin is dissolved in hot water (80°C) and added to the aerated blend while continuing the whipping process for 10–15 seconds. The mousse may be stored frozen or refrigerated.

The vanilla product is made by blending cream cheese and sugar in Hobart bowl, followed by the addition of condensed milk and cream. The mix is whipped to 25% overrun at 10°C. The gelatin solution obtained by dissolving gelatin in hot water (80°C) is then blended along with vanilla flavor for 10–15 seconds. The mousse is ready for packaging and storage under frozen or refrigerated conditions.

PUDDING WITH PARTICULATES (RICE AND TAPIOCA)

In the production of these products, milk is cooked with appropriate rice or tapioca. The starch liberated during the cooking process produces gel-type texture. Since there are particulates in these puddings, sterilizing conditions and processes are distinctly different from those used in smooth single phase puddings where starches per se are used. We will discuss rice pudding in detail. For tapioca pudding, rice is substituted with tapioca grains.

Rice Pudding

Selection of Rice. Taste, appearance, and nutritional criteria decide the type of rice required for preparing rice pudding. Types of rice generally used include long grain rice, medium grain rice, and parboiled rice. Rice-based dairy desserts, traditional or otherwise, may be prepared from rice in different

forms, namely, whole or broken grains and grain fractions. Rice pudding containing whole rice grains is popular. Since different types and varieties of rice have widely varying cooking characteristics, they exert considerable influence on the quality of the product as well as its texture and other sensory attributes. In the production of rice pudding, the cooking parameters are critical for the product quality. Evaluation of raw rice before and after cooking would provide important guidelines for selecting the suitable rice. In general, rice containing high amylopectin content is avoided because amylopectin fraction of starch leads to retrogradation and the rice texture becomes too firm during refrigerated storage of the pudding.

Cooking Characteristics of Rice. Depending on the end use, rice processing may involve any of a wide range of unit operations such as cooking, flaking, roasting, and puffing. However, cooking or heating in the presence of water is the most common process. The amount of water needed to cook rice is an indication of the approximate increase in size of the grain (swell). However, rice grain increases more than twice in volume, even if it is cooked in only twice its volume of water. Long grain rice tends to swell more than short grain rice, and parboiled rice swells less than nonparboiled rice. Long grain rice gives intact kernels in rice pudding than short grain rice. Short grain rice in ultrahigh heat processing becomes less firm than long grain rice, but may develop excessively mealy and grainy texture during refrigerated storage. Brown rice or unpolished rice swells somewhat less than polished rice. Rice swells more when cooked in milk than in water. Texture of the cooked rice is an important determinant of its acceptability. It is particularly significant in relation to rice pudding.

The production of rice pudding for ambient storage and marketing poses technical challenge. The problems associated with thermal processing of heterogeneous products such as milk-rice could be circumvented by adopting newer technologies such as microwave heating, scraped surface heat exchangers, and sterilization in retortable pouches. In-package thermal processing offers certain unique advantages such as flexibility of scale of operation and obviates the need for aseptic packaging. Further, thermally processed and packaged desserts are expected to have adequate shelf life to facilitate their transportation and shipping over long distances for widespread marketing.

A process has recently been reported (Aneja et al., 2002) for in-package cooking and sterilization of rice pudding in retort pouches with the objective to enhance its shelf life at ambient temperature (Fig. 17.3).

Sterilization is done in steam-air environment, using a rotary retorting system, employing a constant rotation of 2 rpm. Retort temperature and pressure are set at 121.1°C and 15 psi, respectively. An overpressure of 30 psi is maintained to prevent bursting of pouches. Concentrated milk, raw rice (washed and soaked at 30°C/30 minutes), and sugar are filled in retort pouches (size 200 mm × 170 mm) having a configuration of polyester, aluminum-foil, and 350-gauge cast polypropylene. The product obtained by this process exhibits proximate composition of 6.21% fat, 10.75% protein, 28.67% total solids, 0.74% ash, and 10.97% carbohydrates. The product had a shelf life of more than four months at 37°C.

Physicochemical Characteristics. During rice pudding manufacture, physical changes in the rice grains being cooked are observed, leading to rheological changes in the milk-rice mixture. Gelatinization of starch is the most prominent change during cooking of rice. It results in uptake of water by the starch granules accompanied by expansion of the network of the starch molecule chains. Swelling and softening of the gelatinizing rice grains are concomitant processes. Gelatinization of rice may take place even during soaking if the soak-water temperature is high enough. When the rice is milled, the cells of the grain are fractured and some of the embedded starch grains are exposed on the surfaces of the individual particles. If the rice grains are agitated during cooking, many of these starch granules are dislodged. These thicken the liquid around the individual particles of the rice, resulting in cooked rice with individual pieces embedded in a thick starch paste of a gooey consistency. Further, during the gelatinization or cooking process, water is believed to disrupt the protein matrix, part of the protein bodies, and the starch granules. Amylose diffuses out of the granules which eventually collapse. This seems to enable a starch-protein interaction. Rheological properties of rice pudding serve as important acceptability parameters. The body and texture of rice pudding improves when the fat content of milk is increased from 3 to 5%. The presoaking of rice at 30 or 50°C for 30 minutes improves the body and texture. Viscosity of the milk-rice mixture increases logarithmically with increase in total solids. The overall textural acceptability of the product is

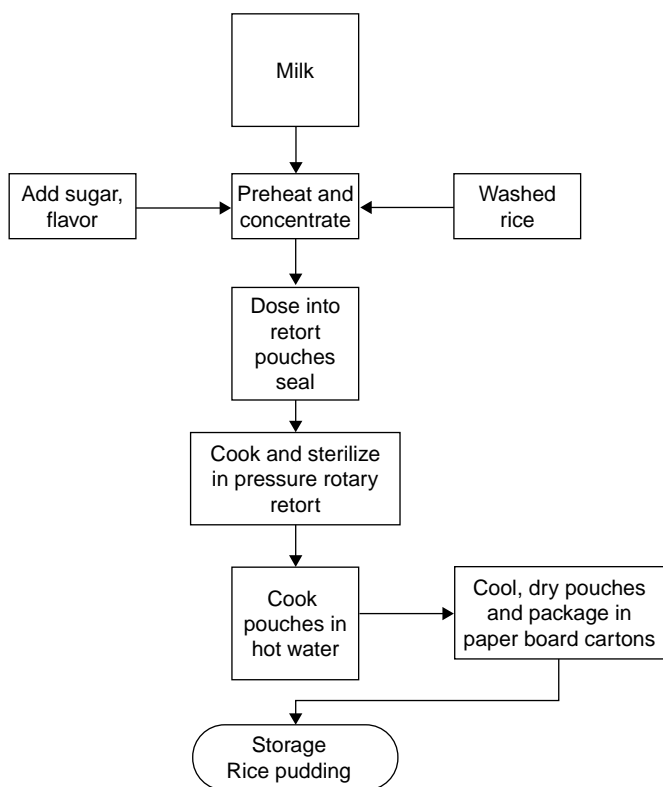


Figure 17.3. Flow chart for manufacturing long-life rice pudding.

determined by both the liquid phase viscosity and cooked grain tenderness.

Processing Procedures

Batch process. In a typical procedure, rice pudding is prepared by immersion of presoaked rice (5–6% by weight of milk) in simmering milk followed by sugar addition (6–8%) and heating the mixture further till the rice softens and shows signs of gelatinization, leading to substantial thickening. Rice grains (broken or whole) are exposed to saturated steam at a pressure higher than atmospheric pressure (>0.03 bar) in an autoclave for sufficient time to gelatinize a major portion of starch. The rice grains are then mixed with milk. Production process for in-can sterilized creamed-rice dessert involves short-grained rice releasing starch on cooking (Aneja et al., 2002). Homogenization of milk improves the product quality. Gradual heating up periods and agitation were necessary during autoclaving to prevent localized overheating. The product exhibits some browning and age thickening during its shelf life of 12 months.

Long grain, medium grain rice, milk, sugar, eggs, vanilla, and salt are added to a batch cooker. Heat processing involves a temperature of 88°C and holding a period of 30–40 minutes, with gentle stirring. The product is hot-packaged in clean environment, vacuum-sealed, and cooled to $4\text{--}5^{\circ}\text{C}$. The ESL product is marketed refrigerated and has a shelf life of 60–75 days.

Ultra-high temperature process. Rice pudding has also been manufactured employing a scraped surface heat exchanger. A typical formulation for aseptically packaged sterilized pudding is given in Table 17.16.

Rice and milk are pumped into scraped surface heat exchanger system, heated up to 115°C , held for 26 minutes in a holding tube, and then cooled down to 80°C in another cylinder. The rice pudding is transferred to a sterile buffer tank, and then filled into form-and-fill plastic containers in aseptic conditions. This process gives better shelf life by subjecting the product to more rigorous heating regime enough to kill the microbial vegetative cells as well as their

Table 17.16. Suggested Formulation of Aseptically Processed Rice Pudding

Ingredient	Weight (%)
Water	62.7
Heavy cream	12.0
NFDM	5.4
Sugar	11.2
Rice	7.0
Flavor/color	0.6
Carrageenan	0.5
Egg yolk	0.34
Sodium stearoyl lactylate	0.2
Trisodium pyrophosphate	0.04
Sodium acid pyrophosphate	0.02

NFDM, nonfat dry milk.

Adapted from Lo et al. (2000) and Budinoff (2003).

spores. To retain freshness, the product is refrigerated.

QUALITY CONTROL ESSENTIALS

1. All the incoming dairy and other ingredients should be checked to insure their conformation to specification with respect to chemical composition, microbiological quality, and physical specifications
2. During the manufacturing processes, insure heating of pudding mix accords starch an optimal cooking. If starch is undercooked, its appearance is cloudy, texture is thin, and viscosity is too low. On the contrary, overcooked starch loses viscosity and acquires cohesive long texture. Optimally cooked starch is clear and maintains stability with good viscosity and heavy bodied short texture.
3. Depending on the UHT equipment being used, processing conditions should be optimized. Preheating in direct steam heating system and in scraped surface heat exchanger is not required, whereas for plate heat exchangers preheating at 65–76°C (no holding period) is recommended. Homogenization is not recommended for direct steam heating equipment. For plate heat exchanger equipment, homogenization at 20 kg/cm² helps to avoid sandiness in the product. Similarly, homogenization at 75 kg/cm² for scraped surface heat exchanger is given by Rapaille and Vanhemelrijck (1998). According to the authors, effective heat treatments are also specific for the type of processing equipment. In general, optimum heat treatment for direct steam heating equipment is 142°C for 5 seconds, plate heat exchanger requires 140°C for 10 seconds, and the scraped surface heat exchanger equipment needs 138°C for 5 seconds.
4. Sensory evaluation of fresh and stored samples for appearance, flavor, and texture should be done. A score sheet should be developed and the terms used to describe the attributes should be fully understood by the sensory panel. Any free liquid on the surface or sides of the product should be noted.
5. The viscosity, hardness, and cohesion values for dairy desserts have been suggested in Rapaille and Vanhemelrijck (1998). Viscosity measurements were made at 20°C with a Haake Rotovisco-meter type RV2, using the coaxial measuring unit MV1. Viscosity is expressed as MPa·s as calculated from the shear stress at a shear rate of 25/minutes. The textural values (hardness and cohesion) were assayed on Instron Universal Testing machine, model 1140, using a compressor load cell-type CBM 50 N and applying the General Texture Profil Method. The authors recommend that for creamy smooth pudding, the viscosity (MPa·s) should be 750–1,000, hardness (N) should be 0.5–1.0, and cohesion should be 0.7–0.9. For gelled-type desserts, the recommended viscosity, hardness, and cohesion values should be 650–1,000, 1.0–1.5, and 0.5–0.7, respectively.
6. Microscopic examination (under polarized light) by counting the number of nongelatinized starch particles in the heat-processed pudding mix should reveal the degree of the starch gelatinization. Also, the degree of starch degradation can be evaluated under microscope by observing the nature of swollen starch granules.
7. The overrun is a measure of the volume of air/gas whipped into the aerated product. Percent overrun in yogurt mousse is 50–80%, whereas in whipped dairy mousses, it ranges from 100 to 130%. For whipped creams, it is in the range of 200–250%. Since overrun has a profound effect on the texture and mouthfeel of whipped products, it is essential to control the overrun in the product.
8. Shelf life studies of the products stored at 4–5°C as well as under abuse conditions should be monitored on systematic basis. The samples should be evaluated for flavor and for textural degradation as indicated by deleterious changes in viscosity, shortness, gel strength, lumpiness, creaminess, and syneresis.

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18

Role of Milk and Dairy Foods in Nutrition and Health

Ramesh C. Chandan and Arun Kilara

Introduction
Definition of Milk
Chemical Composition of Milk
Role of Milk Constituents in American Diet
Nutritional Role of Milk
 Milk Proteins
 Milk Fat
 Lactose
 Minerals
 Vitamins and Some Other Minor Constituents
 Health Benefit of Dairy Products
References

INTRODUCTION

This chapter provides basic information relative to nutritional and physiological aspects of consuming milk and dairy products. To comprehend the role of various constituents of milk in nutrition and health, it is necessary to understand the chemical and physical compositions of milk. Therefore, a brief review of the chemical composition is also provided.

DEFINITION OF MILK

For commercial utilization in the United States, milk is defined as the whole, clean lacteal secretion of one or more healthy cows properly fed and kept, excluding the milk obtained within 15 days before calving and 3–5 days after. Clearly, this would exclude colostrum, the milk secreted immediately after birth of the calf. It is interesting to acknowledge that colostrum is now an established functional food containing high levels of antibodies for protection of the newborn calf.

CHEMICAL COMPOSITION OF MILK

From chemical standpoint, milk is a complex fluid containing at least 100,000 separate molecules and chemical entities.

In the dairy and food industry, the composition of milk is generally described in terms of its commercially significant constituents, namely, milk fat and nonfat solids (NFS) or milk solids-not-fat (MSNF). The MSNF comprises protein, lactose, and minerals. As per U.S. Food and Drug Administration standards of identity, milk is “the lacteal secretion practically free of colostrum, obtained by complete milking of one or more healthy cows, which contains not less than 8.25% MSNF and not less than 3.25% milk fat.” The major constituents of milk of mammals important in human nutrition are given in Table 18.1.

In terms of physical structure, milk is an opaque, white heterogeneous fluid in which various constituents are held in multi-dispersed phases of emulsion, colloidal suspension, or solution (Table 18.2). The phase of the milk constituents influences the nutritional and physiological characteristics of the milk constituents.

ROLE OF MILK CONSTITUENTS IN AMERICAN DIET

Milk is composed of unique nutrients designed for nutrition and well-being of the young of a particular mammalian species. Its composition varies with nutritional needs of the neonates. It provides complete nutrition for the newborn. Milk has been described as

Table 18.1. Composition of Milk Commonly Used for Consumption in Various Regions of the World

Animal	Water (%)	Protein (%)	Fat (%)	Lactose (%)	Ash (%)
Cow	87.3	3.4	3.7	4.8	0.7
Buffalo	82.7	3.6	7.4	5.5	0.8
Goat	87.7	2.9	4.9	4.1	0.8
Sheep	80.7	4.5	7.4	4.8	1.0
Horse	88.8	2.5	1.9	6.2	0.5
Camel	86.5	3.1	4.0	5.6	0.8

Adapted from Fox (2003) and Aneja et al. (2002).

nature’s nearly perfect food for humans of all ages as it provides vital nutrients including proteins, essential fatty acids, minerals, and lactose in balanced proportions. Nutrient composition of milk is illustrated in Figure 18.1.

Leading nutrition experts recognize milk and milk products as important constituents of a well-balanced and nutritionally adequate diet. In this regard, milk products complement and supplement nutrients available from grains, legumes, vegetables, fruits, meat, seafood, and poultry.

Milk and dairy products are considered nutrient-dense foods because they supply a high level of nutrients relative to their caloric value. As shown in Table 18.3, dairy products contribute a vital proportion of nutrients in American diet.

The data in the table show that in the year 2000, milk and dairy products (other than butter) contributed just 9% of the total calories provided by all foods consumed. Concomitantly, milk and milk foods furnished 72% of the calcium, 33% of the phosphorus, 26% of the riboflavin, 22% of the vitamin A, 20% of the vitamin B₁₂, 19% of the protein, 16% of the magnesium, 9% of vitamin B₆, 6%

Table 18.2. Physical State and Particle Size Distribution in Milk Facilitating Nutritional and Physiological Effects

Physical State	Type of Particles	Size, Diameter (nm)
Emulsion	Fat globules	2,000–6,000
Colloidal dispersion	Casein-calcium phosphate	50–300
	Whey proteins	4–6
True solution	Lactose, salts, and other substances	0.5

of the folate, and 5% of the thiamin in American diet.

NUTRITIONAL ROLE OF MILK

Various milk constituents of nutritional significance are proteins, fat, lactose, and minerals. Milk proteins have a high nutritional value because of their high-essential amino acid content. Thus, they complement and balance the amino acid composition of relatively lower quality of several vegetable proteins in human diet.

They act independently and synergistically with each other. The role of major and minor constituents in human nutrition is intertwined with newly discovered physiological benefits

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 U.S. food supply of protein.

Milk protein contains all the nine essential amino acids which human body cannot synthesize. Therefore, the essential amino acids must be furnished by the diet. Table 18.4 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 nonessential 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 18.5 shows the data to support this claim.

The major proteins of milk are casein and whey proteins in the ratio of 80 to 20. Casein

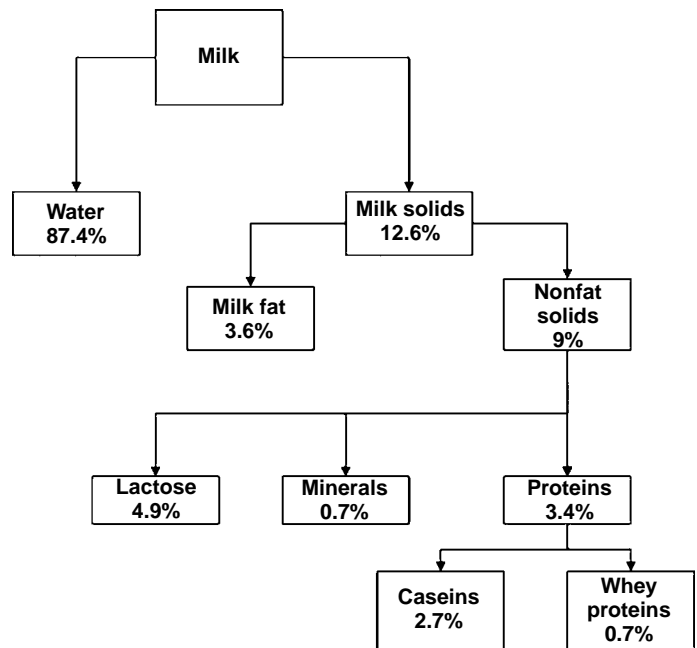


Figure 18.1. Major nutrient composition of milk.

further consists of various fractions including α_{S1} -casein and α_{S2} -casein, β -casein, and κ -casein (Table 18.6). Also shown are the major whey proteins of milk.

A number of proteins found in milk are now recognized for their physiological activity. Among them are immunoglobulins, lactoperoxidase, lactoferrin, folate-binding protein, insulin-like growth factors (IGF-1 and IGF-2), mammary-derived growth factors (MDGF-I and MDGF-II), transforming growth factors ($TGF_{\alpha 1}$, $TGF_{\alpha 2}$, and TGF_{β}), fibroblast growth factors, and platelet-derived growth factors. Peptides derived from milk proteins are gaining recognition for their biological/functional role. For example, evidence points to physiologically active

- glycomacropptides from κ -casein,
- phosphopeptides and caseinomorphins derived from caseins,
- immunomodulating peptides,
- platelet-modifying peptide,
- angiotensin-converting enzyme (ACE) inhibitor peptide (that lowers blood pressure),
- calmodulin-binding peptides, and
- bactericidal peptides from lactotransferrin (Otter, 2003).

Table 18.3. Contribution of Nutrients by Dairy Products (Except Butter) to the United States Per Capita Food Supply of Nutrients, 2000

Nutrient	Percent Contribution
Energy	9.1
Protein	19.4
Fat	11.8
Carbohydrate	4.5
Calcium	72.2
Phosphorus	32.7
Zinc	16.8
Magnesium	15.8
Potassium	18.1
Sodium	32.6
Iron	1.9
Riboflavin	26.3
Vitamin A	22.1
Vitamin B ₁₂	20.3
Vitamin B ₆	8.7
Folate	6.2
Thiamin	4.7
Ascorbic acid	2.5
Vitamin E	2.4
Niacin	1.2

Source: Gerrior et al. (2004).

Table 18.4. Distribution of Amino Acids in Cow's Milk

Amino Acids	Concentration in 2% Reduced Fat Milk (mg/Serving of 244 g)	Milk Solids-Not-Fat ^a (mg/g)	Recommended Daily Allowance for Adults ^b (g/day)
1. 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 ^c	213	9.5	1.3
Phenyl alanine ^d	412	18.34	2.3
Threonine	385	17.12	1.4
Tryptophan	120	5.34	0.35
Valine	571	25.39	1.68
2. Nonessential			
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	—

^a Based on 8.67% MSNF for whole milk.

^b Values calculated for 70 kg male.

^c Total Sulfur containing amino acids methionine + cysteine.

^d Total aromatic amino acids, phenylalanine + tyrosine.

Adapted from Aneja et al. (2002) and Miller et al. (2007).

Table 18.5. 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 concentration	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 milligrams of limiting amino acid in 1 g of test protein and milligrams of the same amino acid in reference requirement pattern multiplied with true digestibility.

True digestibility: $I(F - f)/I$, where I = 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 $BV \times PD$.

Adapted from Schaafsma and Steijns (2000); Southward (2002); and Miller et al. (2007).

Table 18.6. Major Proteins of Cow's Milk and their Fractions

Casein Fractions	Concentration (g/L)
α_{s1} -casein	10.3
α_{s2} -casein	2.7
β -casein	9.7
κ -casein	3.5
C-terminal β -casein fragments	0.8
Whey Protein Fractions	Concentration (g/L)
N-terminal β -casein fragments	0.8
β -lactoglobulin	3.4
α -lactalbumin	1.3
Immunoglobulins	0.8
Bovine serum albumin	0.4

Adapted from Schaafsma and Steijns (2000) and Chandan (2007).

Both caseins and whey proteins of milk possess physiological and biological properties. The biological properties of milk proteins are summarized in Table 18.7.

Caseins

They are further divided into α_{s1} -, α_{s2} -, β -, and κ -fractions, which along with whey proteins, β -lactoglobulin and α -lactalbumin, are gene-derived proteins synthesized in the mammary gland. All these proteins are heterogeneous and exhibit genetic polymorphs. There are 2–8 genetic variants differing from each other in 1–14 amino acids. The variants may have impact on the protein concentration and functional properties of milk. The γ -fraction originates from β -casein by the effect of native proteolytic enzyme of milk.

Caseins display distinctive structure, charge, physical, and biological properties as well as nutritional role. The interaction of various caseins and calcium phosphate contributes to the formation of large colloidal complex particles called casein micelles. Hydrophobic interactions with calcium phosphate and sub-micelles seem to be involved in the formation of micelles. Micelle composition consists of 63% moisture and the dry matter consists of 92–94% protein and 6–8% colloidal calcium phosphate. Other associated salts are magnesium and citrate.

The caseins are phosphorylated proteins, containing 1–13 phosphoserine residues. κ -Casein exists in

as many as nine glycosylated forms. It contains two cysteine molecules per molecule. As a result of disulfide bond formation, it can exist as polymers of 2–8 units. Similarly, α_{s2} -casein also contains two cysteines and exists in a dimeric form.

Casein micelles contain α_{s1} -, α_{s2} -, β -, and κ -casein in the ratio of 3:1:3:1. Most of the fractions α_{s1} -, α_{s2} -, and β -casein are located in the interior of micelles with κ -casein predominantly wrapped around the surface of the micelle. Casein fractions in the interior of micelle are sensitive to calcium and become insoluble in the presence of calcium. However, κ -casein is not sensitive to calcium and thereby keeps the micelles-containing calcium-sensitive caseins intact and suspended in aqueous phase. κ -Casein is a protein with hydrophilic carbohydrate moiety (sialic acid) that extends into aqueous phase. This arrangement further lends stability to the micelle. Casein micelles are stable under most heating, homogenization, and other dairy processing conditions.

Caseins possess certain distinctive amino acid makeup which impacts their processing and functional properties. They are rich in apolar and hydrophobic amino acids, namely, valine, leucine, isoleucine, phenylalanine, tyrosine, and proline. The apolar amino acids are normally insoluble in water, but their nature is balanced by phosphate groups so that caseins exhibit some solubility. Methionine and cysteine, the sulfur-containing amino acids are relatively low in caseins. This fact impacts their nutritional deficiency. On the contrary, the content of essential amino acid, lysine, is high. In human diet, the high lysine content is helpful in complementing and balancing the low-lysine plant proteins. The ϵ -amino group of lysine present in caseins interacts with the aldehyde group of lactose at elevated temperature, leading to the formation of brown pigments. This also explains browning of heat-sterilized milk and nonfat dry milk during extended storage.

The high proline content results in low α -helix and β -sheet in their secondary structure, giving them ability for more proteolytic degradation and enhanced digestion (Otter, 2003).

Among the minor caseins of milk, γ -casein is the C-terminal fragment of β -casein, a product of attack by natural proteolytic enzyme plasmin. The N-terminal residue is the proteose-peptone fraction. These hydrolysis products of β -casein occur at a range of 3–10% of the total casein content of milk. The stage of lactation and health status of the cow affect their concentration.

Table 18.7. Bioactivity of Milk Proteins

Protein	Physiological Effect
Caseins	<p>Precursors of bioactive peptides; calcium and phosphorus carrier. Bioactive peptides of caseins like casomorphins lower gut motility and gastric emptying rate, enhance uptake of amino acids by intestinal epithelial cells, and enhance immune response and phagocytic activity.</p> <p>Casokinins (Ace-I) enhance blood flow to intestinal epithelium.</p> <p>Isracidin, casecidins, and kappacin exhibit bactericidal activity against pathogenic organisms. κ-casein enhances the growth of bifidobacteria in the gastrointestinal tract.</p>
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, increase serotonin production in brain, improve mood, and decrease cortisol level.
Immunoglobulins A, M, G ¹ , G ²	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, antithrombic activity, anticarcinogenic, antioxidant, and iron absorption. Enhances proliferation of intestinal epithelial cells, humoral immune response, and growth of bifidobacteria in gastrointestinal tract.
Lactoperoxidase	Antimicrobial, antioxidant, and immune-enhancing properties.
Lysozyme	Antimicrobial, synergistic with immunoglobulins, and lactoferrin.

Adapted from Chandan (1999); Gobetti et al. (2007); Harper (2000); and Hoolihan (2004).

Biologically Active Peptides Derived From Casein. Peptides derived from caseins are biologically active and display significant extra nutritional attributes for maintaining normalcy of physiological functions in human subjects.

Table 18.8 lists bioactive peptides originating from caseins and whey proteins.

Bioactive peptides are generated during digestive processes in the body and during the fermentation processes used in fermented dairy foods (Gobetti et al., 2007). These peptides are inactive in the native proteins but assume activity after they are released from them. They contain 3–64 amino acids and display largely hydrophobic character and are resistant to hydrolysis in the gastrointestinal tract. They can be

absorbed in intact form to exert various physiological effects locally in the gut or may have systemic effect after entry into circulatory system. Casomorphins derived from milk caseins are known to be opioid agonists while casoxins act as opioid antagonists. The opioids have analgesic properties similar to opium. Casokinins are antihypertensive (lower blood pressure), casoplatelins are antithrombotic (reduce blood clotting), and phosphopeptides are mineral carriers.

Casein phosphopeptides may aid in bioavailability of calcium, phosphorus, and magnesium for optimum bone health. They may also be helpful in preventing dental caries. They may also have a role in secretion of entero-hormones and immune enhancement. Scientific evidence suggests a role of casein

Table 18.8. Some Functional Properties of Bioactive Peptides Derived from Caseins and Whey Proteins

Peptide	Origin	Function
Casomorphins	β -caseins	Opioid agonists and ACE inhibitory/hypotensive
Casoxins	κ -casein	Opioid agonists
Casokinins	α -and β -caseins	Antihypertensive
Casoplatelins	κ -casein	Antithrombotic
Casecidin	α -and β -caseins	Antimicrobial
Immunopeptides	α -and β -caseins	Immunostimulants
Phosphopeptides	α -and β -caseins	Mineral carriers
Glycomacropeptide	κ -casein	Suppress appetite, antiplatelet, anticancer, antihypertensive, prevent dental caries, gingivitis, antiviral, antibacterial, and bifidogeni
β -Lactorphin	β -lactoglobulin	Opioid agonist, inhibition of ACE
α -Lactorphin	α -lactalbumin	ACE inhibitory
Lactkinins	β -lactoglobulin α -lactalbumin	ACE inhibitory (antihypertensive)
Lactoferricin	Lactoferrin	Antimicrobial activity
Lactoferroxins	Lactoferrin	Opiod antagonist

ACE, angiotensin-I-converting enzyme.

Adapted from Harper (2000); Pihlanto-Leppala (2003); Saxelin et al. (2003); and Aimutis (2004).

peptides in regulation of blood pressure. Conversion of angiotensin-I to angiotensin-II is inhibited by certain hydrolyzates of casein and whey proteins. Since angiotensin-II raises blood pressure by constricting blood vessels, its inhibition causes lowering of blood pressure. This ACE-inhibitory activity would therefore make dairy foods a natural functional food for controlling hypertension. A commercial ingredient derived by the hydrolysis of milk protein has an anxiolytic bioactive peptide with antistress effects. Psychometric tests and measurement of specific hormonal markers have displayed antistress effect.

The glycomacropeptide released from κ -casein as result of proteolysis may be involved in regulating digestion as well as in modulating platelet function and thrombosis in a beneficial way. It is reported to suppress appetite by stimulating CCK (cholecystokinin) hormone. Consequently, it may be a significant ingredient of satiety diets designed for weight reduction. Furthermore, this peptide may inhibit binding of toxin in the gastrointestinal tract.

Some miscellaneous bioactive factors are being discovered. Specific proteins for binding vitamin B₁₂, folic acid, and riboflavin may assist in

enhancing bioavailability from milk and other foods. Fat globule membrane protein called butyrophilin is a part of the immune system. Other growth factors in milk may help gut repair after radiation or chemotherapy.

Whey Proteins

Whey proteins, also called serum proteins, provide an excellent balance of essential amino acids. The amino acid profile resembles that of skeletal muscle, making them effective in stimulating protein synthesis in adult muscle. Thus, they preserve muscle mass and enhance health. Whey proteins enhance fat loss. Whey proteins contain more branched chain amino acids than any other protein, which are metabolized (to generate energy) in the muscle rather than in the liver. This property makes them suitable for use by athletes engaged in endurance sports like marathon racing. In general, it has been shown that whey proteins enhance humoral immune response. The sulfhydryl containing amino acids, cysteine, and glutathione are related to immune response. The branched chain amino acids stimulate muscle

glutamine synthesis. Glutamine is involved in immune function. Glutathione formation is facilitated by high cysteine content of whey proteins, which in turn controls significant antioxidant defenses and immune function in the body.

Whey proteins are especially rich in cysteine. β -Lactoglobulin contains 33 mg of cysteine per gram protein, while α -lactalbumin and bovine serum albumin contain 68 and 69 mg cysteine per gram protein, respectively. The $-SH$ compounds are also involved in quenching toxic-free radicals.

In sports nutrition, the high content of arginine and lysine amino acids of whey proteins may help in stimulating release of growth hormone leading to increase in muscle mass and decline in body fat. Furthermore, glutamine protects immune system from decline caused by overtraining.

Whey proteins consist of β -lactoglobulin and α -lactalbumin, bovine serum albumin, immunoglobulins (mainly, IgG1, IgG2, and IgM), lactoferrin, proteose-peptone, and a number of diverse enzymes.

β -Lactoglobulin. This major whey protein of milk displays the presence of four genetic variants. Besides, the two genetic variants namely, A and B, variants C and D have also been reported. β -Lactoglobulin is rich in sulfur amino acids, containing five cysteine residues. It exists as a dimer linked by 1-3 disulfid bonds. It is a fairly heat-labile protein. β -Lactoglobulin stimulates lipolysis and thereby generates rancidity. It also acts as a carrier of vitamin A. The large numbers of lysine residues can result in lactosylation and accompanying changes in physical properties of the protein.

α -Lactalbumin. In human milk, α -lactalbumin is a major protein but in cow milk it is second in preponderance to β -lactoglobulin. Three genetic variants are reported, but Western cow contains variant B only. This protein is rich in tryptophan and sulfur amino acids cysteine and methionine. There are four disulfide in the molecule and it exists as a monomer. α -Lactalbumin has 54 amino acid linkages identical with the enzyme lysozyme. It is a glycoprotein as well as a metalloprotein. One mole of calcium is bound to each protein molecule, which confers heat stability on α -lactalbumin. This protein has been shown to possess a physiological role in the synthesis of lactose in the mammary gland. It is a component of lactose synthetase along with uridine diphosphate-galactosyl transferase, catalyzing the transfer of galactose to glucose to form lactose.

α -Lactalbumin is a calcium-binding protein enhancing thereby calcium absorption. It is an excellent source of essential amino acids such as tryptophan and cysteine. Tryptophan regulates appetite, sleep-waking rhythm, and pain perception. Cysteine is important in functions of $-SH$ compounds.

Immunoglobulins. There are five major classes of immunoglobulins, namely, IgA, IgD, IgE, IgG, and IgM. Their concentration is very high (100 g/L) in first two to three milkings after calf birth, but falls to 0.6–1 g/L soon after. Immunoglobulins are antibodies synthesized in response to stimulation by specific antigens. These offer nonspecific humoral response to Gram-negative enteric and aerobic bacteria. Accordingly, they provide passive immune protection to the newly born calf. The basic structure of all immunoglobulins is similar, which is composed of two identical light chains (23,000 Daltons) and two identical heavy chains (53,000 Daltons). The four chains are joined together by disulfid bonds. The complete molecule has a molecular weight of about 180,000 Daltons. The antigenic sites are located at the $-NH_2$ terminal of the respective chain. Of the five immunoglobulin classes, IgG is the predominant fraction of milk, comprising of about 90% of the total colostrum immunoglobulins. Relatively smaller concentrations of IgM and IgA are also present in progressively decreasing amounts.

The immunoglobulins of milk are important for imparting immune defense for the host. IgG1 is a major component. Other fractions are IgG2, IgA, and IgM, all of which provide passive immunity.

A number of colostrum products are being marketed for use as functional ingredients in foods. Colostrum contains several functional constituents including antibodies, lactoferrin, lactoperoxidase, cytokines, and growth factors. The antibodies act as antimicrobial agents against infection from rotavirus (which causes diarrhea), *Escherichia coli* (which causes food poisoning), *Candida albicans* (which causes yeast infection), *Streptococcus mutans* (which causes dental caries), *Clostridium difficile* (which causes antibiotic-associated diarrhea), *Cryptosporidium parvum* (which causes food poisoning), and *Helicobacter pylori* (which causes ulcer and gastritis). Colostrum stimulates active immune system by enhancing the activity of natural killer cells and phagocytes. The colostrum powder is manufactured by a special drying process to insure activity. Milk protein concentrate prepared from the milk of hyperimmunized cows is claimed to relieve joint

pains of arthritis by complementing the body's naturally occurring anti-inflammatory substances.

Bovine Serum Albumin. As the name indicates, this protein originates from blood and during synthesis in the udder spills into milk. It is a large molecule with binding ability for fatty acids and metals.

Lactoferrin/Lactotransferrin. This is a glycoprotein which displays strong tendency to bind ionic iron due to the presence of two metal binding sites. The average lactoferrin content of 0.32 mg/mL has been found for cow milk. The molecular weight of lactoferrin varies between 73,700 and 74,000 Daltons. Lactoferrin displays very strong chelating tendency for ionic iron and forms a salmon red color pigment. Lactoferrin is a single peptide chain, with two lobes, each of which is capable of binding iron. Iron-free form of lactoferrin is known as apolactotransferrin, which is colorless in appearance. Lactoferrin displays activity against several Gram-positive and Gram-negative bacteria, yeasts, fungi, and viruses. It particularly shows strong inhibitory effect toward Gram-negative enteropathogenic bacteria by virtue of its ability to bind free ionic iron, which is essentially required for the growth of enteropathogenic microorganisms. In this way, lactoferrin has a role in nonspecific defense of the host against invading pathogens. Apart from the antibacterial effect in the gut of calf, a nutritional role in iron metabolism has also been ascribed to lactoferrin. Its iron-binding characteristic aids in enhancing iron absorption. It stimulates and protects cells involved in host defense mechanism by controlling cytokine response.

Biologically Active Peptides from Whey Proteins.

As shown in Table 18.8, a number of peptides derived from whey proteins exert physiological activity. The bioactive peptides of whey proteins have been shown to exert positive influence on body composition, satiety, and weight management. In addition, the bioactive peptides have ACE-inhibitory activity.

Milk Enzymes. Milk is a repository of a variety of enzymes. Over 60 indigenous enzymes have been reported in cow milk. They are either associated with milk fat globule membrane (xanthine oxidase, sulfhydryl oxidase, and γ -glutamyltransferase) or with skim milk serum (catalase and superoxide

dismutase) or with micelles of casein (plasmin and lipoprotein lipase).

Lactoperoxidase breaks down hydrogen peroxide and exerts an antibacterial effect. Therefore, it is considered as a natural preservative. Lysozyme displays antimicrobial activity against Gram-positive bacteria and acts by lysis of cell walls. It has also been suggested that lysozyme may have an indirect effect on the defense systems as an immunomodulator through the stimulation of immune system by the breakdown products of the cell wall (peptidoglycan). Nutritional role of the milk enzymes is questionable since they are destroyed by heat treatment used in milk processing.

MILK FAT

Milk fat in freshly secreted milk occurs as microscopic globular emulsion of liquid fat in aqueous phase of milk plasma. The composition of milk fat is given in Table 18.9.

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.

Physiological Effects of Milk Fat Components

The functional properties of milk fat are attributed to its fatty acid makeup. 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 atherogenic activity of saturated fat like milk fat, medical

Table 18.9. Constituents of Bovine Milk Lipids

Lipid Fraction	Milk (g/L)	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

Table 18.10. Milk Fat: Fatty Acid Profile

Fatty Acids	Common Name	Weight (%)
C _{4:0}	Butyric	3.8
C _{6:0}	Caproic	2.4
C _{8:0}	Caprylic	1.4
C _{10:0}	Capric	3.5
C _{12:0}	Lauric	4.6
C _{14:0}	Myristic	12.8
C _{14:1}	Myristoleic	1.6
C _{15:0}	—	1.1
C _{16:0} (branched)	—	0.30
C _{16:0}	Palmitic	43.7
C _{16:1}	Palmitoleic	2.6
C _{17:0}	—	0.34
C _{18:0} (branched)	—	0.35
C _{18:0}	Stearic	11.3
C _{18:1}	Oleic	27.42
C _{18:2}	Linoleic	1.5
C _{18:3}	Linolenic	0.59

authorities have restricted its intake. The industry has responded with the introduction of low-fat and nonfat dairy foods. It is interesting to note that the milk fat contains 7–8% short-chain fatty acids (C₄–C₈) which is a unique characteristic of milk fat (Table 18.10).

Milk fat exists in an emulsion form in milk making it highly digestible. Also, milk fat contains 10% short- and medium-chain fatty acids. Their 1:3 positions in the glyceride molecule allow gastric lipase with specificity for these positions to predigest them in the stomach itself. Butyric acid, a characteristic fatty acid of milk fat, is absorbed in the stomach and small intestine and provides energy similar to carbohydrates. Medium-chain fatty acids are transported to the liver for rapid source of energy. The fatty acids lower the pH for facilitating protein digestion. At the same time, acid barrier for pathogenic activity is enhanced. Free fatty acids and monoglycerides are surface tension lowering agents, thereby exerting an anti-infective effect.

Butyric acid is liberated from milk fat by lipase in the stomach and small intestine. It may exert beneficial effect on the gastric and intestinal mucosa cells. In the colon, butyric acid is formed by fermentation of carbohydrates by the resident microbiota. Butyric acid in that location works as a substrate for colon cells and confers anticancer properties.

Milk fat is a concentrated form of energy. It is responsible for 49% of the total energy of whole milk, 35% of the energy of 2% fat-reduced milk, and 21%

Table 18.11. A Summary of Health Effects of Certain Milk Fat Constituents

Constituent	Physiological Effect
Butyric acid	Reduce colon cancer risk.
CLA	Modulate immune function; reduce risk of cancer (stomach, colon, breast, and prostate).
Sphingolipids	May reduce risk of colon cancer.
Stearic acid	May modulate blood lipids to reduce risk of cardiovascular and heart disease.
Triglycerides	May enhance long-chain fatty acid and calcium absorption.

CLA, conjugated linoleic acid.
Adapted from Hoolihan (2004) and Chandan and Shah (2006).

of the energy of 1% low-fat milk. Fat protects organs and insulates body from environmental temperature effects. Milk fat functions as a source of fat-soluble vitamins A, D, E, and K and essential fatty acids, linoleic, and linolenic acids. The essential fatty acids are not synthesized by human body in required amounts. They must be supplied by the diet. Arachidonic acid with four double bonds is present in traces. Its precursor is linoleic acid. Omega-3-linoleic acid and its products EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are also present in trace but significant amounts. The positional location of individual fatty acids in the triglycerides is not random. In fact, the *syn*-1 and *syn*-2 positions on the glycerol molecule are mainly occupied by myristic (C_{14:0}), palmitic (C_{16:0}), stearic (C_{18:0}), or oleic acids (C_{18:1}). The *syn*-3 positions contain butanoic (C_{4:0}), hexanoic (C_{6:0}), or oleic (C_{18:1}) acids.

A summary of some beneficial effects of milk fat is shown in Table 18.11.

Bovine Milk Fat Globule Membrane. The envelope surrounding the milk fat contains several health-promoting factors. Both protein and non-protein constituents are potential nutraceuticals. The phospholipids constituents of the fat globule membrane protect against colon cancer, gastrointestinal pathogenic organisms, Alzheimer disease, stress-related diseases as well as depression. The functional properties of phospholipids are further discussed in section “Phospholipids.” Some other membrane components

exhibit health properties, including inhibition of growth of cancer cells, lowering of serum cholesterol, inhibition of *H. pylori* (involved in stomach ulcers), and suppression of β -glucuronidase of the intestinal *E. coli*. In addition, the membrane-situated enzyme xanthine oxidase acts as a bactericidal agent. Butyrophilin is a possible suppressor of multiple sclerosis.

Conjugated Linoleic Acids. These are a class of fatty acids found in animal products such as milk and yogurt. Rumen flora synthesizes conjugated linoleic acid (CLA). Milk fat is the richest source of CLA (2.4–28.1 mg/g of fat). The range of CLAs in most dairy products is 2.5–9.1 mg/g of milk fat. The CLA isomer found in dairy products is predominantly the most biologically active CLA identified as *cis*-9, *trans*-11-18:2 isomer. It is also called rumenic acid. The CLAs have been demonstrated to exhibit potent physiological properties. The CLAs are strong antioxidant constituent of milk fat and may prevent colon cancer and breast cancer. CLAs have been shown to enhance immune response. Prostaglandin PGE-2 promotes inflammation, artery constriction, and blood clotting. CLAs may reduce risk of heart disease by reducing levels of prostaglandin PGE-2. Studies have indicated that CLA may increase bone density, reduce chronic inflammation and normalize blood glucose levels by increasing insulin sensitivity.

Trans Fatty Acids. Scientific evidence has suggested that trans fatty acids (TFA) generated from unsaturated fatty acids in vegetable oils as a result of hydrogenation process increase the risk of coronary heart disease (CHD). Trans fats raise the serum cholesterol fraction associated with risk for developing CHD and the low-density lipoprotein fraction (LDL) and simultaneously lower the protective cholesterol fraction [high-density lipoprotein (HDL)]. The main trans fatty acid is identified as elaidic acid, 18:1, *trans*-9. In the milk of cows and other ruminants, the TFAs are naturally present at 3.56% level in milk fat, but their chemical structure and physiological effects are completely different in comparison to the TFA from hydrogenated vegetable oils. The TFAs of ruminant milk are trans vaccenic acid (18:1, *trans*-11) and rumenic acid (18:2 *cis*-9 *trans*-11). They are CLAs. Several studies have shown that TFA from milk fat do not increase the risk of CHD. The label declaration of TFA is mandated by U.S. Food and Drug Administration regulations.

The threshold level is 0.5 mg per serving. Milk contains 0.22 mg per serving and shows zero TFA on the label. In fact most milk products are below the threshold level.

Phospholipids. A number of factors influence the unique phospholipids content of milk. The total phospholipids content of cow's milk is approximately 2–5 mg/100 mL. Approximately 60% of the milk phospholipids are located in the fat globule and the membrane surrounding the fat in the globule. The rest of the phospholipids are present in skim milk membranous material.

Phospholipids are important because they are constituents of membranes and have a role in cell interaction with antibodies, ions, and hormones. They display surface active properties and act as emulsifiers. Accordingly, they may improve fat absorption in the gastrointestinal tract. They are considered to protect gastric mucosa and may even extend protection from pathogenic microorganisms. Major components of phospholipids are phosphatidyl choline (35%), phosphatidyl ethanolamine (32%), sphingomyelin (25%), phosphatidyl inositol (5%), and phosphatidyl serine (3%) (Taylor and MacGibbon, 2003).

Sphingolipids are hydrolyzed in the gastrointestinal tract to ceramides and sphingoid bases, which help in cell regulation and function. Studies on experimental animals show that sphingolipids inhibit colon cancer and reduce serum cholesterol as well as elevate HDL, the good cholesterol. They could protect against bacterial toxins and as well as infection.

Cholesterol. Dietary total fat, saturated fat, and cholesterol are postulated to increase serum cholesterol, particularly LDL, which is a risk factor for CHD. However, not all fatty acids cause elevation in total serum cholesterol. Stearic acid has neutral effect, while lauric, myristic, and palmitic acids increase blood cholesterol levels. Individuals vary greatly in their blood lipids response to dietary fat and saturated fat. Also, it should be recognized that genetic factors are also responsible for synthesis of cholesterol in the liver and bulk of the serum cholesterol is controlled by genetic causes.

In general, typical cholesterol content of whole milk (3.25% fat) is 10.4 mg/100 mL. It corresponds to 3–4 mg/g fat. In view of consumer preference for low-fat and nonfat dairy products, fat reduction is accompanied by cholesterol reduction. By separating fat from milk, an 80% reduction in cholesterol

content can be achieved in skim milk. Thus, nonfat milk/skim milk shows residual cholesterol level of 5 mg/8 fl oz serving. In contrast, whole milk (3.25% fat), 2% reduced fat milk, and 1% low-fat milk respectively contribute 24, 20, and 12 mg of cholesterol/8 fl oz serving.

LACTOSE

The major carbohydrate of milk, lactose monohydrate, ranges from 4.8–5.2%. Lactose stimulates the absorption of calcium and magnesium. It has relatively lower glycemic index of 46 as compared to 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. A compound formed from lactose in heated milk products is lactulose. Heated milk contains up to 0.2% lactulose. Since lactulose is not a digestible ingredient, it acts somewhat like a soluble fiber. Lactulose is generally used for the treatment of constipation and chronic encephalopathy. Some recent data indicate that lactulose may enhance calcium absorption in the intestine. It stimulates the growth of *Bifidobacterium bifidum* and is thereby beneficial in establishing useful microflora in the gut.

Lactose Intolerance

Lactose intolerance is characterized by symptoms of bloating, flatulence abdominal pain, and diarrhea after consuming milk and milk products. Certain ethnic groups in the United States, namely, a majority of African-Americans, Asians, and Southern Europeans experience such symptoms. It is mainly attributed to discontinuation of milk consumption after infancy as seen in the dietary pattern of such groups. In such cases, due to absence of lactase in the diet, epithelial cells intestinal mucosa tends to lose the ability to secrete lactase enzyme. Lactase is a nonpersistent enzyme in certain individuals, resulting in distressing symptoms following milk intake. Lactase or β -galactosidase is a key enzyme in digestion of lactose. It catalyzes the hydrolysis of lactose to glucose and galactose which are rapidly absorbed. Temporary absence of lactase from the digestive tract may also be exhibited during or following enteric infections, when the surface lining of intestinal mucosa is damaged due to invasion of enteropathogenic bacteria. So when milk is consumed following weaning for several years or after enteric infection, symptoms of lactose intolerance (maldigestion) may be seen. In the

past, it was believed that lactose maldigesters should avoid the use of milk and dairy products in their diet. However, new evidence shows that most nonpersistent lactase individuals can tolerate two cups of milk spread over a day or a cup with meals. They may also choose lactose-reduced products, low-lactose products, or lactase supplements. In case of lactose malabsorption, the symptoms are ameliorated by consuming yogurt containing live and active cultures. Yogurt and fermented milks furnish the enzyme lactase to assist in digesting lactose. Lactose-reduced milk and ice cream products are also available. This way lactose-maldigesters have the vital nutrients of dairy foods, especially, calcium, protein, vitamins, and minerals available to them.

MINERALS

Milk is an excellent source of minerals. The mineral content of milk is given in Table 18.12.

Table 18.12. Major and Minor Minerals of Cow's Milk

Major Mineral	Mean (mg/100 mL)	Range (mg/100 mL)
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	Mean (μ g/100g milk)	Range (μ g/100g 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

Adapted from Fox (2003) and Chandan and Shah (2007).

Functional Role of Calcium and Other Minerals

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). One of the primary functions of calcium is to provide strength and structural properties to bone and teeth. Lack of adequate calcium intake particularly during growth phase leads to osteoporosis or brittle bones in later life. It is also important in teeth development.

Calcium is involved in muscle contraction (including heartbeat), blood coagulation, enzyme reactions, stimulation of hormonal secretions, and 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.

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, 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.

VITAMINS AND SOME OTHER MINOR CONSTITUENTS

In order 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

Table 18.13. Vitamin Concentration of Milk

Vitamins	Concentration in 100 mL Milk
A	40 µg RE
D	4 IU
E	100 µg
K	5 µg
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

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 18.13.

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 our 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 help in reducing falls in the frail elderly.

Vitamin E is an antioxidant. Vitamin K is present in milk but its dietary nutritional role is probably minor.

Milk is an important source of dietary B vitamins. They are stable to various heating and processing conditions milk is normally subjected to. Vitamin B₁, thiamin, is a cofactor in carbohydrate metabolism. Vitamin B₂ 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 B₆ is critical in protein metabolism. Folic acid acts as a growth factor and is involved in DNA synthesis. Vitamin B₁₂ 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 nonheme iron. It provides resistance to infections. However, vitamin C content of milk is relatively low.

HEALTH BENEFITS OF DAIRY PRODUCTS

Traditionally, the nutritional role of milk has been linked to the supply of essential and nonessential 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 benefit 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.

Weight Management

Epidemiologic evidence indicates that the risk of weight gain was 67% lower in group consuming the most dairy products as compared with the group consuming least dairy products (Huth et al., 2006). Human clinical trials with obese subjects have indicated that during caloric reduction, higher loss of body weight and body fat takes place on diet containing adequate calcium from calcium supplements. Interestingly, this loss is relatively much more when the identical level of calcium is contributed by dairy sources. It appears that the weight and fat loss by calorie-controlled diet is caused by changes in metabolic partitioning of dietary energy, maximized by intake of calcium (1,200–1,300 mg/day) from dairy products. Another mode of calcium action in reducing weight in low-calorie diet may be interaction of calcium with dietary fat to form soap-like material in the gut, which is not absorbed and is subsequently excreted out. The distribution of body fat loss is also different in that only calorie reduction diet resulted in loss of 19% body fat around trunk area. However, the loss around trunk area was 50% in the diets containing calcium from supplement sources and was 66% in the diet containing dairy products (Zemel et al., 2005). It should be emphasized that the fat loss with added dairy products is conditional upon the restriction of total caloric intake. When diet is high in

calories, adding dairy products to diet would not help in weight and fat loss. Accordingly, the weight and fat loss should occur when three to four servings of dairy products are part of low calorie diet.

Type 2 Diabetes

Management of this disorder is critical for controlling the resulting complications, namely, cardiovascular disease, renal failure, blindness, and amputations. Dietary intervention for controlling this disorder is being researched. Several studies have shown that lifestyle modification including diet are important factors in prevention of type 2 diabetes. Epidemiological studies have indicated that the consumption of high levels of dairy foods significantly reduces the risk of developing type 2 diabetes in men and women.

CLA a natural component of milk fat has been reported to improve human body's poor regulation of insulin when administered for 8 weeks or longer. It improves the utilization of glucose resulting in its lowering in the blood of type 2 diabetic patients.

Bone Health and Prevention of Osteoporosis

Osteoporosis is related to progressive loss of bone tissue which results in skeletal weakness and consequently leads to bone fractures after age 50 in later part of life. Optimum bone growth needs adequate amounts of dietary protein, vitamins A, C, D, and K as well as minerals calcium, phosphorus, magnesium, copper, manganese, zinc, and fluoride. These nutrients should be supplied by a variety of food sources. Dairy products are significant sources of calcium, phosphorus, and magnesium in American diet. Optimum bone development requires adequate dietary calcium to achieve peak bone mass and dairy foods play a significant role in bone health. Bone is composed of 50% protein and approximately 50% calcium phosphate crystals. Bone tissue process is in dynamic state throughout life. The older bone breaks down and newer bone tissue is constantly replacing it. During childhood and teen years, provided that adequate nutrients are available, bone formation exceeds by far the breakdown (resorption) phase. Approximately 85–90% of peak bone mass is achieved by age 18 in girls and age 20 in boys. Bone mass in early stages of life is a good determinant of bone strength in later stages of life. In adult life, the rate of bone resorption is in equilibrium with that of bone formation. If bone resorption exceeds bone formation, a net loss of bone mass occurs, leading to

bone porosity, fragility, and fractures. Certain types of bone cells involved in bone remodeling cease to function resulting in progressive loss of bone mass. Patients with osteoporosis are administered medications to activate remodeling cells while they are encouraged to consume calcium, vitamin D, and other dietary nutrients. The 2005 Dietary Guidelines recommend three cups of low-fat or nonfat milk or equivalent from dairy products for adults to help maintain bone health.

Osteoporosis occurs more commonly in women than in men. Key elements in developing osteoporosis are genetic factors, age, physical activity, and nutrient intake. In women, it is aggravated by menopause (loss of estrogen). Nevertheless, there is a consensus that maximizing peak bone mass by adequate calcium, phosphorus, and other minerals from dairy and food sources in early stages of life is necessary for retaining higher bone mass and reduce the risk of bone fractures in later phase of life.

Hypertension Control

Calcium and potassium are known to be associated with beneficial regulation of blood pressure. Milk and dairy foods are good sources of these mineral and are now regarded to exert an antihypertensive effect. Various population studies have shown relationship between dietary intakes of 1,000 mg/day or more and 40–50% reduction in the prevalence of hypertension. Randomized clinical trials have shown that calcium from food sources is more effective than calcium from supplements. The blood pressure lowering effect by dairy foods is not related solely to calcium content, but is related to other minerals like potassium, magnesium, and vitamins, proteins and essential fatty acids as well (Huth et al., 2006). In this regard, milk proteins are documented to be sources of bioactive peptides generated by proteolytic enzymes involved in digestive processes or by the action of lactic cultures used in fermented dairy products. These peptides lower the blood pressure by ACE-inhibitor activity as shown in animal and human trials to reduce the blood pressure from hypertensive range to normal levels (Wrick, 2007). The scientific evidence shows beneficial role of including three servings of dairy foods in diets high in fruits and vegetables in preventing hypertension.

As discussed in previous sections, milk proteins are precursors of bioactive peptides capable of inhibiting activity of ACE. This enzyme regulates blood pressure, electrolyte, and fluid balance in the body.

ACE converts inactive angiotensin I hormone into angiotensin II, which constricts vascular smooth muscle leading to elevation of blood pressure. An inhibitory effect of the peptide derived from milk protein results in lowering of blood pressure. In this regard, whey protein hydrolyzates are particularly cited for their blood pressure lowering ability (Miller et al., 2007).

Cancer

The effect of diet on the risk of cancer is being clarified and 30% of all cancers are related to our diet. It is known that certain components of foods when consumed in excessive amounts may promote cancer. Such diet items are calories, alcohol, and fat. On the contrary, some food components are preventative. Dairy foods provide some of the preventative nutrients. In this regard, calcium and vitamin D are recognized to be beneficial against colon cancer. Studies have shown that the increased consumption of dairy foods which are rich in calcium and vitamin D should reduce risk of colon cancer. Dietary calcium is found to normalize hyperproliferation of colonic epithelium cells in individuals at risk of colon cancer.

It has been fairly established that CLA and vaccenic acid found in all dairy foods confer desirable anticarcinogenic and antiatherogenic effects. CLA is also beneficial in glucose and fat metabolism in type 2 diabetic subjects. Since fat content of popular dairy foods is low, their CLA content is also low. The isolated CLA may have nutraceutical role in the future. In any case, more research is needed to establish the efficacy and safety of CLA in human subjects.

Enhancement of Health Properties by Culturing of Milk

Fermented milks like yogurt are enhanced functional foods since they contain nutrients of milk as well live cultures and products of metabolic activities of starter microorganisms in the product. Particularly, they contain live and active cultures in significant numbers to effect physiological benefit to the consumer. Bacterial mass content and the products of the lactic fermentation further distinguish yogurt from milk.

Probiotics, Beneficial Cultures, and Functional Ingredients. Probiotics may be defined as a food or supplement containing concentrates of defined strains of living microorganisms that on ingestion in certain doses exert health benefit beyond inherent

basic nutrition. Probiotics and associated ingredients might add an attractive dimension to cultured dairy foods for effecting special functional attributes.

Several hundred bacterial species inhabit the distal regions of the human digestive tract. Their population exceeds the total cell counts in human body. Functions of the intestinal flora include modulation of cell growth and differentiation, antagonistic activity against pathogens, and other infections, immune stimulation of gut-associated lymphoid tissue, reduction of blood lipids, and biosynthesis of vitamins. In healthy individuals, the colonies of diverse gut bacteria exist in equilibrium. Factors such as stress, age, gastrointestinal disturbances, and antibiotic therapy are known to upset the balance of gut microflora and resultant malfunctioning of their digestive and metabolic effects. Probiotics help in restoring the balance.

Milk is an excellent medium to carry or generate live and active cultured dairy products (Chandan and Shah, 2006; Shah, 2006; Vasilijevic and Shah, 2007). Fermentation adds an attractive dimension to cultured dairy products for augmenting current demand for functional foods. The buffering action of the milk proteins keeps the probiotics active during their transit through the gastrointestinal tract. In general, worldwide consumption of fermented milk products has increased due to their high nutritional profile, unique flavor, desirable texture, and remarkable safety against food-borne illness.

Cultures associated with health benefit are yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*), other lactobacilli, and bifidobacteria (Chandan and Shah, 2006). Yogurt organisms possess several documented health attributes. To bolster probiotic function, most commercial yogurt is generally supplemented with various species of *Lactobacilli* and *Bifidobacterium*. Yogurt starter bacteria, *Lb. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*, are also now considered to act as probiotics because of their health-promoting effects. In particular, yogurt bacteria have been scientifically demonstrated to assist in lactose digestion, reduce or prevent diarrhea episodes, and strengthen immune defenses of the host. They are now reported to persist and remain viable throughout the human gastrointestinal tract. The continuous ingestion of live products insures abundant numbers to maintain their functional status. Even with intestinal isolates such as *Lb. acidophilus*, it is necessary to dose regularly rather than to assume that a few doses will allow the organisms to colonize the gut permanently.

Reported health benefit (Pannell and Schoenfluss, 2007) associated with yogurt and probiotic cultures include the following:

- Stimulate immune system
- Improve digestive regularity and alleviate constipation
- Reduce symptoms of lactose intolerance
- Reduce risk factors for colon cancer initiation
- Increase calcium absorption
- Reduce risk of cardiovascular disease by lowering serum cholesterol
- Alleviate inflammatory bowel disease and irritable bowel syndrome by restoring normal balance of gastrointestinal microflora
- Enhance resistance to colonization by pathogenic organisms
- Reduce symptoms of eczema and skin diseases associated with gut immune system

Yogurt and fermented milks are commonly supplemented with various functional ingredients. In addition to probiotics, they include prebiotics and fiber, plant sterol esters, and omega-3 fatty acids, minerals, and vitamins.

Prebiotics and fiber include polysaccharides (for example, inulin), oligosaccharides [fructooligosaccharides (FOS), galactooligosaccharides (GOS)], and lactulose. They are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth/activity of one or limited number of bacteria in the colon that have the potential for improving the health of the host. They stimulate probiotic organisms like bifidobacteria and lactobacilli. Prebiotics provide nutrients for colonic bacteria, thereby sustaining balance among various populations of enteric bacteria. Health attributes of prebiotics reported in the literature are prevention of colon cancer initiation, stimulation of immune response, providing barrier against pathogenic organisms, prevention of adhesion of pathogens and toxins, render systemic effects on blood lipids, and help in mineral absorption. In this aspect, their action resembles probiotics.

Plant sterol esters are added for their cardiovascular benefits. Clinical trials have demonstrated that the yogurt and fermented milk containing this functional ingredient lowers serum cholesterol levels in humans.

Omega-3 fatty acids are postulated to reduce the inflammation and found in oily fish, flax, walnuts, canola oil, and pumpkin seeds. They include linolenic acid which is abundant in flax seeds. Others are EPA

and DHA that are obtained from fish oils. They are required for good heart health and normal human growth and development and are implicated in brain function and development, asthma, immune function, arthritis, skin, and hair health.

Fortification with minerals and vitamins is done to provide a significant dose (33–50%) of required intake of daily minerals and vitamins.

Probiotic Supplements. Several preparations of dry probiotics are available in the form of tablets, powder, or capsules. They contain organisms from the genera *Lactobacillus*, *Enterobacter*, *Streptococcus*, and *Bifidobacterium*. These genera are important members of the gastrointestinal microflora and are all reportedly beneficial. The strains of lactic acid bacteria used in probiotics preparations are intestinal isolates such as *Lb. acidophilus*, *Lb. casei*, *Lb. rhamnosus*, *Enterococcus faecium* and *Bifidobacterium bifidum* and other bifidobacteria. In general, the probiotic preparations are supplemented with enzymes, anti-inflammatory compounds, specific amino acids, colostrums, and chelated minerals.

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19

Product Development Strategies

Vijay Kumar Mishra

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INTRODUCTION

Product and process developments are some of the key activities most companies continuously engage in to remain commercially viable, competitive, profitable, and eventually grow as a business entity. Through these activities, consumers are offered products, which satisfy a particular need. Most food companies consider innovations in new products and processes as key to the success in the marketplace and create wealth. New product development (NPD) process is both a research method and a business strategy (Earle, 1997a). History of food industry development is replete with examples of both product and process innovations as it has transformed itself from a craft to sophisticated technology-based industry. The examples of innovations by food companies are in the development of infant formula, pasteurization of milk, canning of foods, modified and controlled atmospheric packaging of fruits and vegetables, and

sous vide as innovative processes. Similarly, Yakult's probiotic drink, gluten-free bread, and margarine are examples of product innovations.

Every year thousands of new products are introduced in the market; however, the reality is very few are successful and chances of introducing successful product to the market are decreasing. It is widely acceptable that the success rate is less than 12% (Rudder et al., 2001). Product development is therefore a costly activity given the level of resource commitment by the company and fraught with risks, yet it is essential part of any business enterprise. The success of food product development depends on NPD strategies, organization of product development activities, and market orientation (Harmsen, 1994). For successful product development, Earle (1989) quoted the following recommendations by Booze, Allen, and Hamilton (1982) for companies:

1. New products should be developed internally as means of growth
2. NPD efforts should be well define
3. Necessary resources to these efforts should be committed consistently
4. Approaches that address the company goals and needs must be in place and in use
5. Have very well-define new product strategies

Like other industries, food industry has to anticipate forces that provide opportunities for development of new and innovative products. Urban and Hauser (1993) laid out the following forces that industry should be aware of while new product and process development activity is planned by the company management:

Table 19.1. Common Trends in Consumers Toward Food Purchase

Consumer Trends	Typical Examples
Healthy foods	Cholesterol reducing oils and margarines, milk with added supplements (vitamins and minerals, fiber) iodine enhancing salts, cereals foods containing fiber, fermented drinks, fermented milk with probiotics
Convenience	Microwavable foods, minimally processed foods, easy meals
Natural and fresh	Additive free foods, organically grown foods (fruits, vegetables, meat, poultry)
Varied tastes and flavors	Ethnic foods (Asian, middle east foods)

1. Sales growth, profitability, and share prices continue to be under pressure from forces in financial markets
2. High competition from local as well as global market forces
3. New markets are being sought by organizations to search for focused profit opportunities
4. Shorter product life cycles due to market saturation with similar products
5. Influence of introduction and acceptance of high technology on pressurizing industry to become growth orientated
6. Rapid changes in political and demographic pattern and consumer lifestyle
7. Buyers becoming very selective in decision making due to prevalence of supply over demand
8. Increased participation and control of markets by distribution channels
9. Increased availability of new raw materials and ingredients, for example, genetically modified food ingredients and additives
10. Strict environmental controls
11. Use of alliance as strategic tool in planning corporate success

In addition to above, mergers of companies, higher disposable income available to the consumer, availability of plentiful food, presence of value-added products in the market, shrinking of markets, developments in information management, and policy developments have also influenced how food industries see product and process development to be a key business activity for growth.

It is imperative that food-manufacturing companies follow strategies that maximize their chances of success in the marketplace. Basically, a company is developing new product to satisfy a consumer need and the key facets of product development (PD) are how PD is placed in the company strategy, PD process, knowledge management, and understanding of

the relationship between product and the consumer (Earle et al., 2000).

REASONS FOR PRODUCT DEVELOPMENT

CONSUMER DEMAND

A company is in the marketplace to satisfy consumer demands in a given market. The demands continuously change and a firm understanding of what consumers want is key to successful PD. Today's consumer demands foods which are healthy, easy to prepare, natural, tasty and flavorful, produced with methods that are environmentally safe, and safe. Table 19.1 shows some of the common trends in food purchase. These trends do influence the product category growth and hence PD activity. *Food Technology* journal has published several articles that track the food trends (Mermelstein, 2001, 2002; Sloan, 1999, 2001, 2003, 2005), which have guided both product and process development activities. Sloan (2005) described top 10 global food trends as quick fix drive and go; inherently healthy; fancy that; farm friendly; flavorizing; grazers, low, no, and not-so-much-of; do-it-yourself doctoring; and global gangbusters. Food industry has to take notice of these trends and offer new products to satisfy these consumer demands keeping in mind that company should keep on changing and expanding range of products to suit the customer otherwise its share will be lost to those companies who are willing to innovate.

CHANGE IN THE TECHNOLOGY

Developments in agriculture and animal husbandry, postharvest management, processing, nutrition and health sciences, genetics and molecular biology, information technology, and analytical methods have all helped food industry to market food products

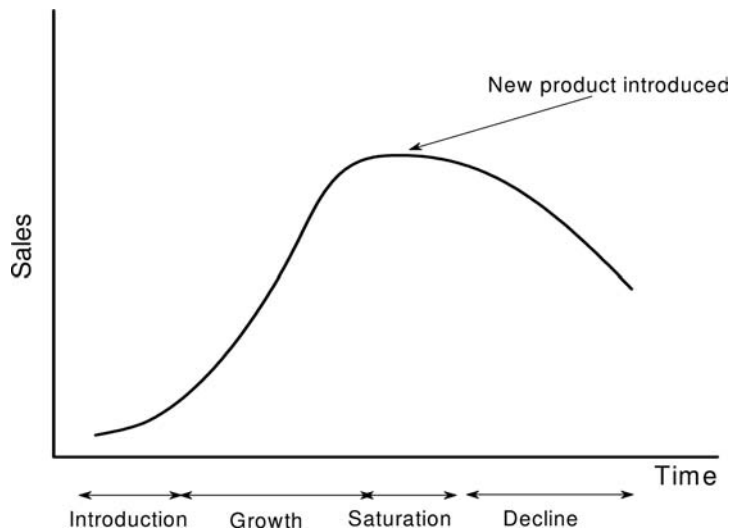


Figure 19.1. A typical product development cycle.

which are safe, and have longer shelf life, superior sensory quality and nutritional value. For example, acceptance of microwave oven in the household has led to development of variety of new products such as microwavable popcorn, cake mixes, and dinners. Similarly, nonconventional heating methods (e.g., ohmic heating), ultrasonic, high-pressure processing have opened up new opportunities to develop products with superior quality (higher nutrient retention, sensory quality, and freshness), as product is not exposed to heat as in the case of thermal processing.

There is general switch to development of processes that are environmentally friendly. For example, the disposal of huge volume of cheese whey has led to invention of new technology to separate whey proteins and lactose-valuable food ingredients, and reuse water. To some in dairy processing industry, whey is no longer a waste product.

There has also been phenomenal increase in the novel ingredients that is revolutionizing food industry. Discovery of artificial sweeteners (aspartame), fat replacers (simplese), modified starch, additives, and emulsifier have led to innovative range of food products.

PRODUCT LIFE CYCLE

Every product has a finite market life beginning with its birth (introduction) and ending into death (withdrawal). How sales progress within this time period

is known as product life cycle. Figure 19.1 shows a schematic of a product life cycle by depicting sales volume as function of time. The cycle can be divided into several phases, which are introduction, growth, saturation, and decline. Introduction follows a period of development that varies enormously. At the time of product introduction to the market, sales are slow and considerable promotional efforts by the company are made to educate the consumer. The company will hold monopoly during this period and with very little revenue being generated as a result of sale due to high cost of production. This is followed by rapid growth in sales, increase in revenue, reduction in the unit costs leading to maximum profit to the company due to repeat purchases made by a satisfied customer. The saturation or maturity phase is characterized by sale volume reaching its peak and slowly leveling off as more competition offerings enter the market and prices tend to drop off. Finally, the decline phase sets in with both sales volume and profit decrease as consumers switch to other products. This signals the end of the product life. In order to remain profitable the companies should manage the product life cycle properly. Above phases in the product life cycle run parallel to market evolution, which includes, crystallization, expansion, fragmentation, consolidation, and termination. There may be three broad strategies to extend the product lifecycle, one to find new use of the product (difficult for food products), second, to find new markets, such as overseas, and, third, to keep on developing and introducing new products

well before the product enters the phase of decline in its lifecycle.

MARKETPLACE

The marketplace is a place where both products and services are exchanged. It is a dynamic environment influenced by wholesalers, retailers, competition, and nature of consumers. Companies spend millions of dollars marketing their products through advertising, sponsorships, and market research. Food marketing also aims at generating demand for a product and making sure that company has a best possible market mix, which is combination of product, price, promotion, and distribution. The success of PD will depend on how well marketing communicates with food technologists.

Marketplace dynamics is influenced by several factors such as consumer demand (healthy, natural), consumer profile (income, lifestyle, education level, socioeconomic background), competition, and selling pattern (e-shopping, catalogue shopping, delis). Supermarket chains do have a huge say in type of the products shelved by these retailers as space on the shelves is sought by food manufacturers.

GOVERNMENT POLICY AND LEGISLATION

The governments influence the business activities of food companies by formulating and implementing the law and regulations to protect the consumer. These laws keep on changing. Operating in a global environment, food industry must continuously update themselves with both local and international laws as they prescribe nutritional guidelines, consumer protection, and product safety measures. For example, at the time of writing of this chapter, there is no health claims allowed in Australia except for folic acid. Reformulation of a product may be needed due to bans imposed by the importing country on certain ingredients or additives used during product manufacture. For example, saccharin and cyclamates are banned artificial sweeteners in the United States and hence low-calorie drinks needed reformulation for U.S. markets.

CORPORATE REASONS

A company would like to grow and increase its profitability by changing and diversifying their offerings to the consumers. The corporate business plan set out financial and growth objectives with a very

specific PD activity that will benefit the stakeholders, which could be the shareholders or private owners. There have been several examples of companies diversifying their portfolio by including new products to their offerings, for example, pharmaceutical companies are beginning to enter into nutraceuticals market.

CLASSIFICATION OF NEW FOOD PRODUCTS

There is no fixed definition of a new product (Rudder et al., 2001). A new product may be (a) an already existing product that has been repacked and given a new name and image, (b) an improved version of an old product that may have new packaging and or brand name, and (c) an original product that serves an unmet need of the consumer. Really new or innovative products are actually rare (Booz, Allen and Hamilton, 1965). New products can be classified into following categories (Fuller, 1994; Kotler and Armstrong, 1991; Linnemann et al., 2006).

Me-too products: These products are similar to the existing ones, but may be marketed by different company. This is the largest category of new food products available in the market. Development of these products can be time-consuming and costly. It requires considerable technical input for development. Example will be margarine marketed by two different companies.

Line extensions: These are new variants of an established line of products by the same manufacture. These products require little development time, expenditure, or effort to develop as there is no major change in processing or equipment, marketing strategy, purchasing skills or raw material sources, and new storage or handling requirements for either for raw ingredients or the final product. An example will be offering a mango-flavored yogurt (new) to strawberry-flavored yogurt (existing).

Repositioned existing products: These are current products for which a new use has been discovered. Development time for repositioned existing product is minimal with no change to the manufacturing process. However, these require a sound marketing strategy with most efforts from the marketing team in promotion. An example will be reposition of margarine containing ω -3 lipids as ingredient or component. Special health benefit associated with consumption of ω -3 provide a marketing advantage.

New forms of existing product: These are products which have taken a new form. An example will be when a company is also offering now liquid soups as powder for convenience. The development of these products involves extensive development time and expertise, manufacturing process and equipment, and change in packaging warehousing and distribution system.

Reformulation of existing product: These are existing products reformulated to improve color, flavor, stability, appeal, and functional characteristics. Reformulation may involve use of new ingredient with different characteristics, such as, fewer calories, and an increase in fiber content. An example will be development of high fiber bread. Normally the design of the process is inexpensive and the development time is short but requires extensive technical expertise.

New packaging of existing products: These products have new packaging to increase shelf life, ease of dispensing or improved shipping properties. An example will be the use of plastic tube to package condensed milk instead of a can. Development of these products may require use of expensive package and equipment for packaging. In some cases the product may need to be reformulated.

Innovative products: These products result from making changes in existing products other than those described above. The degree of innovation dictates the development time and associated costs and extensive marketing may be needed to first educate the consumer. The products in this category are both costly and risky than the other products. An example will be meal prepared by using *sous vide* technology.

Creative or true new products: These products are truly new in nature and have not been in existence. These require extensive development time, marketing effort, expensive equipment, and processes. Because of these requirements, these products are very costly and risky with higher chances of failure. However, if successful, these products may lead to handsome profits. Development of Vegemite by Kraft foods can be an example in this category.

Out of all these categories, it is rare to find creative or innovative products for obvious reasons, yet it is innovative products that are most likely to succeed in the market (Hoban, 1998; Stewart-Knox and Mitchell, 2003) and create wealth for food industry.

PRODUCT DEVELOPMENT STRATEGY

A strategy may be described as a plan, which a company puts in place to gain advantage over its competition in the market. Development of strategy involves use of three key thinking skills, that is, identification of elements and scope, analysis, and synthesis. The basic elements of a strategy for a company are market, stakeholders, and capacity and capability. Product development strategy (PDS) is born out of the business strategy and therefore need to be conceptualized at the time of development of a business plan and is a part of overall corporate strategy. A company must have strategic orientation determined before planning PDS. Table 19.2 lists some of the strategic orientations and key aims of each.

Development of innovative strategies is unique to the company and expected to be flexible enough to change with time. Factors that influence PDS are size of company, management of products and services offered, market type, resources (funds, technological capacity and capability, human), influence of the distribution channels, supermarket chains, and level of competition. Company will use and consider macroeconomic, industry/segment forecasts, and analysis of resources at their disposal and competition, demographic and technological changes for development of strategic objectives for NPDP. Based on these objectives, a positioning statement specifying the consumer group and price aimed at will be generated (Balascio, 1986; Earle, 1989) to guide the development activity.

Innovation (product, process, marketing, and organizational), market, technology and product strategies are used to arrive at a PDS (Fig. 19.2) suitable for the company. There are seven basic innovation strategies based on the key drivers, which are customer, competition, technology, stakeholder, project, resources, culture, and market (Prestwood and Schumann, 2007). These innovation strategies are listed in Table 19.2. Overall innovation strategy for the company will be a convenient blend of above. Examples of product, innovation, and marketing strategies are given in Table 19.3.

PRODUCT DEVELOPMENT TEAM

NPDP is achieved by a team effort. The success of the process therefore depends on how well a team has performed. This requires assuming responsibilities

Table 19.2. Product Development Strategic Orientation

Strategic Orientation	Objective
Time to market	Aim to quickly introduce product to the market. May be technologically driven and require frequent upgrading
Low product cost	To develop least cost or highest value product. Requires additional costs and resources for both product and process development
Low development cost	To minimizing cost of product development. This is the case for those industry who are developing a product on contract
Product performance, technology, innovation	Aim at highest degree of performance, innovation, and technology involvement and therefore incur maximum risks but rewards may be high as well
Quality, reliability, and robustness	Aim at assuring high level of quality and safety. Requires added time and cost of planning, testing, analysis, approval. Very typical of food industries
Service, responsiveness, flexibility	Aim at providing high level of service, responsiveness to customer requirement. Maintain flexibility to cater to new markets and customers. Typical of hospitality industry

Adapted from Crow (2007).

by each of the team player. The team will consist of:

- *Management.* Management establishes the interest of senior management in the project; assures that company’s objectives are strictly adhered to; insures that ideas selected for development fit the corporate or brand image of the company; insure positive atmosphere to check the rivalries that can arise as pressures and deadlines take their toll; and has an ability to assess and manage the strengths and weaknesses of the team.
- *Finance department.* It makes sure that project costs are formulated, monitored, and controlled to within budgetary limits.

- *Legal department.* Legal department plays a major role in controlling the legal aspects of (a) formulation and processing, (b) labeling, marketing and promotion programs, (c) duty of care issues, (d) ownership of intellectual property, (e) interpretation of laws and regulations relating to the product, and (f) impact on the environment.
- *Marketing and sales department.* Their responsibility is to monitor the marketplace for changes that might affect the course of development. Along with the technical department, this department plays a key role during the entire process of the NPD by collecting consumer and market information, developing marketing strategies, monitoring how the product fares compared to the those offered by competition.
- *Warehousing and distribution departments.* Special warehousing or distribution channels (chilled or frozen) or special environmentally controlled storage may be required for some food ingredients or finished products. These departments contribute to the development of handling, storage, and distribution requirements for the new product and assure the marketing department of existence of an appropriate system.
- *Engineering department.* This department assists in the commissioning of both pilot- and full-scale processes once product specification have been formulated and a decision about the launch is taken up by the management. The engineers will design safe processes for plant and full-scale

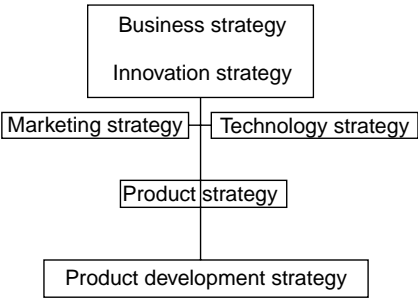


Figure 19.2. Ontogeny of the product development strategy.

Table 19.3. Innovation, Product, and Marketing Strategies

Strategies	Examples
Innovation	Level of innovation or newness (high or low); “let’s stay or keep it to our knitting (limiting the area of innovation)”;
	product differentiation (compare new product to the existing one/s); cost leadership (develop high volume cheap products); fl w (control product introductions over time)
Product	Increasing sales to the existing market (no new product introduction but endeavor to increase sales by branding, improved packaging, improved quality, services); simplifying product design (no new product development but changing the process to reduce the cost of manufacturing); simplifying product lines (removal of slow movers from the range of products); development of new markets/consumers (expand the range of customers); product customization (tailoring the product specification to needs of the buyer); trading up/down (changing the product and sale to new market segment); extending product life cycle (discover new use for the product or expand markets overseas); new product to same market (to offset competition); new product to expanded market (extending product lines and diversification) new product to new market (increasing sales volume of new products to markets which were previously unknown, extremely risky)
Marketing	Customer need; market position (ability to attract customers through advertisement, clever packaging, innovative brand names; health claims); big bomb (target large established category, develop superior products through extensive R&D backed by extensive promotional campaign)

production, investigate the type and source of equipment required, prepare costing, and insure that normal plant operations are not disrupted.

- *Production department.* This department oversees manufacturing or production function of the company and the responsibilities consist of management of all aspects of production, including labor; raw, intermediate and finished material; waste management and quality and safety assurance.
- *Research and development department.* This consists of a team of people who have strong technical background and control the technology aspects of the design and development of the product and subsequent process. The team produces both laboratory and the bench-top models for initial evaluation, establishes specification for raw materials, ingredients, and finished product by carrying out lab tests and translate consumer needs identified by the marketing department into technical variables that define a new product.
- *Quality assurance department.* This department is responsible for assuring that all processing, product, environmental, and worker safety standards are adhered to and that all reasonable and practicable precautions to protect the product

from hazards of public health significance have been taken. In some small food industries, they are part of production department and may act as R&D unit.

FOOD PRODUCT DEVELOPMENT PROCESS

Once PDS has been finalized and company objectives and identification of consumer needs are established, the process of product development can begin. Success of PD hinges on how well the strategy and structure matches the environment that a company works in and an understanding of the consumer needs. Food product development process consists of a series of stages or phases that are followed by a company to convert ideas into a tangible product or service that will satisfy a consumer need. There have been several approaches proposed (Booze, Allen and Hamilton, 1965, 1982; Earle, 1997a,b; Fuller, 1994; Graf and Saguy, 1991; Hanchate, 2006; Hood, 1995; Kotler and Armstrong, 1991; Lord, 2000; MacFie, 1994; Rudolph, 1995; Urban and Houser, 1993) to model PD process and have been reviewed by Rudder et al. (2001). Various stages or phases of PD do vary with the model used for a given situation. A five-step sequential process as proposed by Urban and Houser

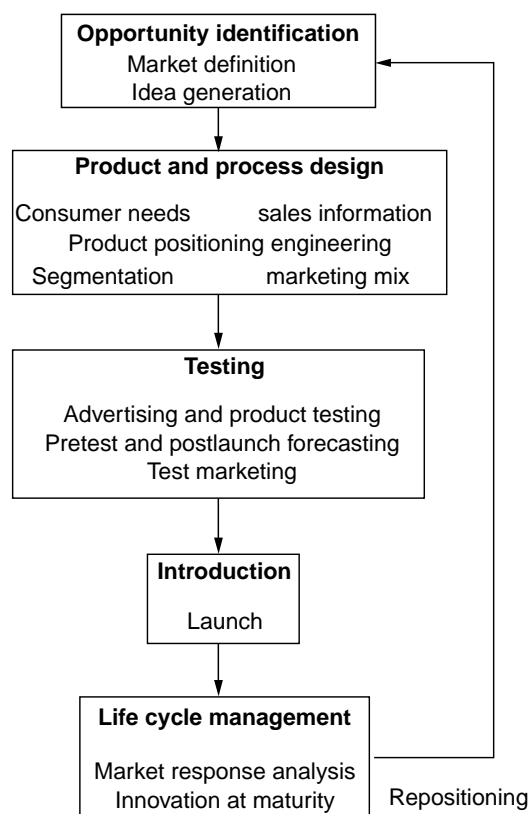


Figure 19.3. New product development process. (Adapted from Costa and Jongen, 2006; Urban and Houser, 1993.)

(1993) is currently accepted as consumer-driven PD process (Costa and Jongen, 2006) and many studies have accepted consumer-driven approach in product development (Jaeger et al., 2003; Mattson and Helmersson, 2007; McWatters et al., 2006; van Kleef et al., 2005; van Trijp et al., 2007). The process is depicted in Figure 19.3.

The first stage, *opportunity identification*, is where strength and capabilities of the company are matched with markets considered to be profitable for NPD activity. This is the step where intended markets are defined and product ideas are generated. Opportunity identification stage requires most input from the marketing team.

The ideas may take a form of a quality attribute, for example, low-energy cake (nutritional), fruit juice containing probiotics (health), a shelf-stable pie (keeping quality), and so on. New food product development ideas can originate from within or out-

side the company. Internally, ideas can come from sales representatives who are in direct contact with consumers and suppliers, know the products marketed by competition; through customer communications as suggestions or complaints to the customer services; in-house product or process experimentations and memory box of employee of the company. Food conferences, exhibits, trade shows, and research symposia, libraries, and literature published by other industries (suppliers) can be external sources to ideas or information that will affect NPD. Food retailing is changing in response to consumers' needs and changing lifestyles. Supermarkets have grown into stores that have mixtures of operations, such as bakeries, seafood counters, butchering, perishable section with huge selection of fresh, trimmed and washed, pre-packed fruits, and vegetables, in addition to selling their own brand of processed foods. Superstores are, therefore, ideal places for collecting new product ideas based on customer needs.

All successful product development starts with the identification of consumer needs and then translating these needs into a product. Starting with preconceived ideas of what a company thinks consumers want have proven to lead to failures (Holligsworth, 1994). Opportunity analysis will involve market analysis, which consists of analysis of consumer, purchase behavior, and competition (Urban and Hauser, 1993). The desirable characteristics of the market targeted will be those having high growth potential, low risk, high return for low investment and can generate economy of scale. Consumers determine what will sell, if and when their needs are satisfied. This information is absolutely essential and is gathered by market research. Market research is an organized and unbiased investigation into factors that influence marketplace and facilitate the decision-making process for a development work. Market research starts early and continues throughout the development process. Consumer focus groups and interviews can be used to collect qualitative information to gather ideas.

Ideas need to be screened to manageable few based on the relative merit, which may be assessed based on response to the following questions/issues. Is the idea feasible within the time frame requested by the marketing? Does the idea meet a perceived need of the consumer adequately? Will a financial sound business plan based on these products stand up to critical analysis? Does the company have appropriate technologic resources? Products that lack marketability may be (a) either so far ahead of their time that they cannot be marketed, or (b) are inferior to

those already on the market, cost more, or exhibit insignificant differences from existing products that consumer is neither able to perceive nor appreciate.

The criteria used for screening ideas and the issues within are related to:

- *Marketability and marketing skills.* Does the marketing department have the ability to market the product to the targeted consumer? Will the product require unique marketing skills? Does the company have sufficient marketing resources within companies?
- *Technical feasibility.* Can development be achieved? How soon can it be done? What will it cost when it is done? Will it be safe? What is the impact on environment? Will there be any legal issues? Technical feasibility can be measured by the (a) chance of success, (b) the time to reach successful development, and (c) the costs of that success.
- *Manufacturing capability.* What are the manufacturing requirements? Where can the raw materials be sourced? Does the company have a manufacturing capability? Will it benefit the company to expand the current manufacturing facility? Can manufacturing be contracted out? Inability to manufacture new products may not necessarily be sufficient reason to cancel ideas for products. Manufacturing and packing capability can always be bought through co-packers within limitations, as profit may be lower and quality compromised. Advantages of contracting out may be if products prove unsuccessful, no capital expenses have been laid out for equipment.
- *Financial criteria.* This may depend on the company's objectives and vary widely depending on whether the product is for an all-year operation versus a product that is only seasonal.

Second stage, *product design*, consists of refining the screened ideas and converting them to some product attributes perceived by the customer. Active involvement from R&D, engineering department, and marketing teams are expected at this stage. Product design is very technical as ingredients, intermediate products, and product itself can be very perishable, may pose health and safety risks, and subjected to regulatory constraints. Specification in terms of product quality, acceptable and available raw materials, recipe, storage, processing, transport and handling variables, packaging requirements, legal requirement, cost and impact on the environment must

be prepared. Design process can be assisted by judicious statistical experimental design and analysis (Hu, 1999; MacFie, 1994) to increase the efficiency. Information on ingredients, packaging materials, and equipment are compiled by R&D department to develop a bench-type prototype. If competitor's product exists in the market then a comparison with the prototype is affected. The prototypes are continuously refined using in-house sensory panels first and followed by consumer panels. This is an iterative process.

During this stage a food technologist needs to consider the following.

Cookbook and chef's recipes normally require extensive revision to be considered as commercial recipes for following reasons:

- Commercial products must be safe with respect to all hazards of public health significance and maintain their quality attributes when they reach the consumer and throughout the entire distribution chain.
- Product cost includes expenses associated with labeling, packaging, transportation, labor, advertising, promotions, quality control, plant maintenance, and plant sanitation. These costs need to be minimized.
- Large volume requirements demand different types and scale of equipment.
- Commercial products must meet the needs and expectations of a price that a consumer is willing to pay for.

The technologist will be involved in product formulation; process development; determination of product storage, packaging and handling requirements and determination of expected shelf life.

In the situation where there is no recipe existing, the food technologist can analyze similar competitive products, read ingredient lists on labels, have the product analyzed by commercial laboratories, or develop their own. The development technologist must be aware of the following issues.

Food Safety

Products must be safe for human consumption. Concern over the safety of food products centers around the following:

1. Presence of pathogens exceeding the standards in the product

2. Presence of toxicants of a biological nature gaining access through the ingredients or formed during processing, for example, enterotoxins, mycotoxins
3. Presence of chemical hazards in the food product, for example, pesticides, herbicides, growth stimulants, fertilizer
4. Presence of extraneous matter, for example, stones, glass fragments, metal pieces, wood
5. Presence of insects and insect parts

Food Spoilage

The new food product developer must understand what are the key factors that cause deterioration of foods and then use appropriate methods that will help in maintaining the stability, safety, and the desired quality attributes throughout the anticipated shelf life. The factors responsible for loss of quality are:

- Biological (respiration, oxidation, fermentation, putrefaction, enzyme associated, microbial activity) lead to softening, discoloration, slime formation, off-flavor, off-odor, loss of texture, color, and structure
- Chemical (oxidation, hydrolysis, Maillard reaction) can lead to discoloration, exudation, off-flavor due to rancidity
- Physical (evaporation, concentration, drying, stress or abuse) lead to separation, clumping, toughening

Following measures are available to the technologist to stabilize foods and insure safety:

- Acidification to $\text{pH} \leq 4.8$
- Lower the water activity of the product
- Lowering the temperature and use refrigerated distribution system
- Use vacuum, modified or controlled packaging
- Use approved preservatives
- Heat treat the product
- Use hurdle approach

Hazard Analysis and Critical Control Point analysis has been an established tool in assuring quality.

On the marketing side, *design stage* involves conducting consumer need analysis, product positioning, sales forecasting, and development of marketing mix, which is a combination of the product, price, promotion, and distribution. The marketing team also assists food technologists in refining the product by providing information about the consumer needs as they evolve.

The product developed needs to be tested to evaluate whether the consumer will accept it. By the time the process reaches *testing stage*, the team is certain that the product design is completed. In the testing stage, tests will be performed carefully to assess product acceptance and promotional campaigns developed by marketing people. Sometimes, consumer is needed to be educated of the benefit the new product provides. Whole marketing mix is put to the test. The product may be tested in simulated environments, for example, by conduction acceptance tests in a superstore or distributing free samples and inviting consumer to provide feedback. These tests are useful in identification of problems with quality and can lead to a conclusion whether product is ready for launch or not.

Sales department mainly leads *product introduction or launch stage*. Company may have to deal with issues relating to intellectual properties at this stage as well. Decision must be made whether to introduce product by a rapid entry or a roll out. A rapid entry is planned to take significant advantage over the competition to derive most returns from the PD activity. Rollouts, however, are considered when the company lacks resources to effect full-scale introduction, unsure of product acceptance by the consumer, unsure of what completion is doing and existence of several markets for the product. The marketing team's endeavor is to establish the product in the market and questions such as how, when, and where to need to be answered. Table 19.4 provides some decision nodes.

The final stage in the PD is *life-cycle management*, which requires monitoring and analysis of market response to the product over its lifetime. Price, promotional strategies and activities, and sales effort may be required to change as the product moves through the maturity phase to retain profitability otherwise company must consider repositioning itself and go back to the drawing board.

Earle et al. (2000) consider NPD process as an industrial research activity consisting of four key stages, *product strategy*, *product design and development*, *product commercialization*, and *product launch and evaluation*. Each stage has critical points, which guide the management to take decisions on the NPD project and products. This will also facilitate identification of key outcome of the project and product.

Lord (2000) described strategic NPD as seven stage processes as suggested by Gill et al. (1996). These stages are: (1) setting PD targets and creating a

Table 19.4. Decision Making for Product Introduction/Launch

Decision	Guidelines
Where to	Markets where targeted consumer exists; no or limited competition; efficient distribution infrastructure exists; presence of more than one retailer; robust but mixed economy
When to	Time when maximum demand likely: for example, Cross heart buns during Easter, cakes and chocolates during Christmas period, ice cream during summer
How to	In store demonstrations; advertisement and promotions (giving away free samples); door-to-door campaigns; through buyers and distributors

portfolio, (2) situation analysis, (3) opportunity analysis, (4) identification of potential product options, (5) establishment of threshold criteria for minimum acceptable performance targets, (6) creating portfolio of new product options in relation to the targets, corporate capabilities to grow and maintain itself, budgetary limits, and customer and competition concerns, and (7) management of portfolio.

It must be emphasized there is no single model of PDP that is ideal for all situations given the complexity. While arguing for the holistic approach to product development, Lundahl (2006) argued for incorporating emotive aspects in developing new food products as this approach changes the focus of the PDP from product features to consumer-product experiences, which lead to successful trial and repeat purchase. This line of thought brings in research into social and psychological experience of consumer. How well does it translate into product success is a valid research question.

IMPROVING CHANCES OF SUCCESS OF NEW PRODUCT DEVELOPMENT

It has been widely recognized that the success rate of new products introduced is low and must be improved to guarantee decent returns for investment in this costly venture. It is obvious that success depends on how well the industry understands the customer needs before attempting to satisfy them with a new product. Before dealing with the subject of improving the rate of success of NPD, there is a need to quantify NPD success in the marketplace. Measuring the performance of the new product is required for organizational and process learning, to quantify benefit for future NPD activity and to gather critical information for marketing, sales, and management so that marketing strategies can be adjusted (Saguy and Moscovitz,

1999). Stewart-Knox and Mitchell (2003) compared the perception of success factors based on studies conducted in the United States (Hoban, 1998), UK (Stewart-Knox et al., 2003), and Denmark (Kristensen et al., 1998, as quoted by Stewart-Knox and Mitchell, 2003). Variations due to the type of market, the indicators used in the survey, and type of survey population were noted (Stewart-Knox and Mitchell, 2003). Table 19.5 provides a comparison of relative importance of the success factors and indicates that for these markets, the main success factors are market and consumer knowledge and involvement of retailers. The U.S. and Danish study also indicated importance of senior management involvement to product success. Besides constraints of the model studies conducted, the differences may be due to differences in the structure, management culture, and the marketing environments (Stewart-Knox and Mitchell, 2003). Differences in the NPD strategies followed by companies in the United States and Europe have been noted previously (Erickson, 1992).

The role of customer in the NPD can never be overemphasized and therefore, product development researchers believe that customer should drive the NPD activities (Bogue and Ritson, 2006; Linnemann et al., 2006; Mattson and Helmersson, 2007; Saguy and Moscovitz, 1999; van Kleef et al., 2005) and food industry must consider structured methodological approach to NPD than a trial and error (Linnemann et al., 2006) to improve product success. Along these directions, approaches have been the use of Goldenberg creativity templates, quality function deployment (QFD), and quality change modeling. Creativity templates are based on the philosophy that radical changes are rejected and minor ones ignored; a successful innovation must be new and easy to comprehend. These templates are defined as (1) subtraction, (2) multiplication, (3) division, (4) task unification and (5) attribute dependency change.

Table 19.5. Comparison of Factors Influencing Success of New Food Product Development

Factor	U.S. survey (Hoban, 1998)	UK (Stewart-Knox et al., 2003)	Denmark (Kristensen et al., 1998)
Uniqueness of the product of high quality	Innovative products most important	Original concepts successful	Product adaptations successful
Market/customer knowledge	Important but second to innovation	Predictive of success	Predictive of success
Involvement of senior management	Important, third to innovation	No association	Predictive of success
Product development/technical synergy	Success factor	No association	No association
Customer/retailer involvement	Success factor	Predictive of success	Predictive of success
Suppliers and other agencies	Success factor	Predictive of success	Predictive of success
Involvement of food technologist	Not assessed	Predictive of success	Predictive of success

Adapted from Stewart-Knox and Mitchell (2003).

The approach is useful in increasing consumer acceptance of complex new food products and underlines the importance of understanding of the food characteristics in PD (Linnemann et al., 2006). There has also been research into using QFD in NPD (Benner et al., 2003; Costa and Jongen, 2006; Costa et al., 2001; Linnemann et al., 2006; Urban and Houser, 1993) to systematically allocate priority to possible solutions to a given set of objectives. While QFD approach is very popular in service industry (Urban and Houser, 1993), its use in food PD has been limited to the use of only first of the four phases due to compositional complexity, presence of interactions difficult to understand and the fact that properties vary over time (Benner et al., 2003; Linnemann et al., 2006). Still the first phase, called the house of quality or product-planning matrix can be useful in facilitating translation of consumer demands to tangible product requirements. Quality chain modeling goes a step further and can assist in incorporation of product requirements arrived at from QFD into a production chain and explore their optimization (Linnemann et al., 2006).

CONCLUSION

Food product development is expensive, time-consuming, and labor-intensive process full of risks,

yet it is being recognized as lifeblood of food industry for growth and development. The success of NPD depends on plethora of factors, most importantly market and consumer knowledge. Given the complexity of understanding variations in food composition and properties, imposition of strict safety requirements, deterioration mechanisms, and changing perception of consumer needs, NPD will continue to be a challenging activity. In order to improve chances of success of NPD, food companies need to develop and follow proactive product development strategies that integrate company structure to its environment (market and technology).

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20

Packaging Milk and Milk Products

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INTRODUCTION

Perhaps as much as any component of the food and dairy processing and distribution system, packaging is basic to the safe delivery of the contained products to consumers. Without packaging, the contained dairy products would not be protected against the environment whose elements are always working to revert contents back to their original components. Contrary to current widely held opinions, packaging's role is to protect the environment by avoiding the monumental quantities of spoiled dairy products which would result from the absence of effective packaging.

This chapter addresses the totality of containment and protection of dairy products from process through consumer use and beyond into the solid

waste stream, but, of course, represents only a brief overview. Readers who require greater depth are referred to various textbooks and articles on the subject. It must be noted that much of the secondary literature on this topic is not in the peer-review journals, but resides on the Internet and in trade journals and analogous publications. The information to be derived from such a probe is generally contemporary and relevant, and of value to readers.

Content of this chapter begins with fundamentals, including the major package materials employed and their principal applications in dairy foods. Dairy packaging operations are described. Because suppliers play a major role in providing packaging components, they are classified and in some instances, identified with no implication of endorsement as a result of inclusion or criticism as a result of omission.

More traditional (from a dairy technologist's standpoint) "packaging" or product/package interactions are reviewed. More traditional (from packaging engineer's standpoint) distribution packaging is also touched. Graphic design, regulations affecting packaging, and the role of packaging solid waste in the environment are discussed. The chapter concludes with an enumeration of dairy product packaging, both current and projected for the future.

FUNDAMENTALS OF PACKAGING

Packaging is the most effective means to protect contained dairy products from their point of manufacture through to their consumption. It is also arguably the most effective means of communication between the dairy products' marketer and the end user of the dairy products since the package's form, dress, and surface

graphics are visible prior to at the instant of purchase decision and as the package is used.

DEFINITION

Packaging is the totality of all elements required to contain the product within an envelope that functions as a barrier between the product and the environment which is invariably hostile to the contained product unless the protection afforded by packaging is present. Environmental insults include temperature, moisture, oxygen, microorganisms, animals, insects, impact, shock, compression, and human disturbances. By totality is meant the package material and its structure and surface graphics, the machinery for linking the product to the package materials, the outer distribution packaging and its associated equipment, the distribution system itself, opening and removing the contents when and where desired, and so forth. The package is the material in its structural form such as a bottle, can, pouch, bag, carton, or case.

The most important definitional element is that packaging is a means of protection for the product while it is in distribution.

PRIMARY PACKAGING

Primary packaging is that which is in intimate and direct contact with the contents. As such it represents the major barrier between the product and the environment. Most, and sometimes all, of the protection against oxygen, microorganisms, light, moisture gain or loss, and so forth, is built into the primary packaging. Among the more common primary packages are metal cans, plastic and glass jars and bottles, plastic tubs and cups, flexible pouches, and paperboard folding cartons.

SECONDARY PACKAGING

Secondary packaging is usually an outer carton or multipacker that enables the consumer to carry more than one primary package of a product at a time. Sometimes the secondary package is an outer carton or wrap to hold just one primary package.

DISTRIBUTION PACKAGING

Sometimes referred to as secondary packaging, distribution packaging is a means of unitizing many primary and/or secondary packages to facilitate the movement of a large multiple of packages as a single

entity. In this manner, the packages are protected and may be economically moved rather than having to move one package at a time. Typical distribution packages include corrugated fiberboard cases and pallets.

PACKAGING TECHNOLOGY

Packaging technology is the application of scientific principles to employ packaging for functional purposes including protection and communication.

GRAPHICS

Graphics represent the external appearance of the package and usually includes dress, form, shape, color, type copy, and so forth, to communicate to the consumer or intermediary some mandated or desired information.

STRUCTURAL DESIGN

Structural design is the three-dimensional shape of the package, cylinder, rectangular solid, tapered cylinder, flat and so forth. Structure is also used to connote the components and order of a multilayer packaging material such as a flexible lamination.

PACKAGE MATERIALS

In an ideal world, a single package material would suffice to protect all dairy products. In this ideal world, a steel can could function in this role, but the size, weight, and economics of a steel can dictate that it not be employed when a less expensive and lighter weight material is available. Because of the nature of materials, it is often necessary to combine different materials to achieve a desired objective, but even the combination of materials is usually less expensive than employing all-metal for many applications. Even metal requires coating, usually plastic, in order to be useful in most dairy packaging applications.

Paper and Paperboard

Paper and paperboard represent the packaging material used by far in largest volume both in the United States and around the world. Because of its origins, it must be combined with other materials to render it effective in packaging applications. Most of this category is consisted of paperboard rather than paper,

with the boundary being 0.010 in., paper being below this gauge dimension and paperboard above.

The two basic types of paperboard are virgin, originating from trees and their wood, and recycled, whose raw material is used paper. Generally, virgin paperboard is cleaner and more uniform and has greater strength per caliper (unit gauge). Further, it accepts barrier material for coating much easier than does recycled paperboard. On the other hand, recycled paperboard may, if desired, have a superior surface for printing. Recycled paperboard has been used as a secondary (nonfood contact) package material for many decades, with the origins of the material being trimmings and scrap from paperboard and corrugated fiberboard converting plants. With the new environmental advocate-driven forces to use "post-consumer" packaging waste, the history of the raw material is unknown, and so food content safety could be compromised by contaminants which cannot be effectively removed in the recycling process.

Because paperboard is moisture sensitive, for dairy products packaging, it is generally necessary to protect the paperboard which then functions primarily as a structural material. Among the coatings used are low-density polyethylene (LDPE) applied by hot extrusion. Polyethylene is an excellent moisture and water barrier to protect the base paperboard.

Paperboard is used in dairy product packaging as the substrate for both gable top and aseptic brick-/block-shaped cartons to contain fluid milk and its analogs. In the aseptic packaging application, it is extrusion laminated with aluminum foil to deliver a long time ambient temperature shelf life.

Probably the major dairy products packaging application of paperboard, however, is in three-layer form in corrugated fiberboard cases used for distribution. The corrugated structure consists of three layers of two outer flat liners of paperboard, usually virgin, plus an inner flute medium, which can be either virgin or recycled. The corrugated structure offers vertical and horizontal compression and impact strength to protect the contents. Increasingly, the printing is being improved to permit the cases to be used as retail displays or even as consumer multipacks.

Metal

Metal is most often used for cylindrical cans which are either thermally processed for microbiological stability or internally pressurized with carbon dioxide as for beer and carbonated beverages. Aluminum is by far the most important metal used for can

fabrication, being the primary metal for beer and carbonated beverage cans, increasingly used for still beverage cans, but only sparsely for food cans except for shallow pet food and fish cans. Almost all aluminum cans are two pieces, that is, a body and an end seamed to the body.

Steel represents the major metal used for food and dairy product cans, usually coated with chrome/chrome oxide and subsequently overcoated with plastic to protect the metal from corrosion and the product from metallic flavors.

Aluminum is also used in very thin or foil gauges as a flexible or semirigid packaging laminant to impart oxygen and/or water vapor barrier to the lamination. In this form, the aluminum must be protected from damage by plastic or paper.

Glass

Glass is historically the oldest packaging material still in use. Glass is the best barrier and by far the most inert to product contents. Further, in appropriate structures, glass has the greatest vertical compressive strength. On the other hand, glass is very heavy per unit of contents contained, is energy intensive to manufacture, and, as is well known, is prone to breakage with impact especially impact after abrasion. Glass may be fabricated into bottles and jars, almost all of which require plastic or metal devices to close.

Plastic

Plastic is the newest packaging material, having been developed in the twentieth century, and having come into prominence only since the 1950s. In actuality, the term *plastic* describes a family of materials related by their common derivation largely from petrochemical sources. Each is quite different in properties relative to packaging requirements, and so no single plastic material is capable of being universally applied. All plastic package materials are characterized by their light weight, relative ease of fabrication, low cost, and ability to be tailored for specific end applications. Together, by weight, all plastics comprise about 20% of package materials, but because of their low densities, protect far larger volumes of contents than any other package materials.

The most commonly used packaging plastic is polyethylene which may be obtained in high, medium, and low densities, with variations available on each of these. Low density polyethylene (LDPE) is tough, flexible, easily formed, and light weight. It

is an excellent water and water vapor barrier, but a poor oxygen barrier. LDPE's most common uses are as flexible pouches and bags, and as the heat sealable extrusion coatings on paper, paperboard, aluminum foil, and other plastics. Thus, LDPE is the exterior and interior coating on gable top fluid milk cartons, the laminant on aseptic brick and block cartons, and the heat seal coating on many cured cheese pouches.

High-density polyethylene (HDPE) is a semirigid, somewhat stiff translucent easily formable plastic. With fairly good heat resistance (for a plastic), HDPE has excellent moisture and water resistance, but very poor gas barrier. HDPE is used to form bottles for milk and drinkable yogurt as well as a wide variety of other products with modest barrier requirements.

Polyester (PET) was available as a specialty film package material for many years, but only since the late 1970s did it enter as a significant package material. A modestly good oxygen and water vapor barrier, in package form after orientation, PET is tough and transparent. PET's major packaging applications today are for carbonated beverage bottles, with other bottles and jars as for small extended shelf life (ESL) dairy beverages, and so forth, thermoformed cups and tubs, and so forth, in the semirigid category being secondary applications at present. PET may also be formed into film that are tough and dimensionally stable, and therefore are quite good to protect aluminum foil or for lidding-type flexible closures.

In oriented film form, polypropylene is an excellent, economic, tough, transparent, high moisture barrier, low gas barrier material which has captured almost the entire quality flexible packaging market. Among the packages being made with oriented polypropylene (OPP) are potato chip pouches, bakery goods overwraps, and candy bar wraps. Because of its relatively high temperature resistance (up to 250°F), polypropylene resin is combined with other higher barrier packaging materials to produce multilayer plastic bottles and cans such as for "bucket-type" cans for microwave reheating. Polypropylene is often injection molded into tubs and cups to contain spoonable dairy products.

Polystyrene is a plastic with relatively poor oxygen and water vapor barrier, but good structural properties. Being inexpensive and easy to form by sheet extrusion and thermoforming or injection molding, polystyrene has been one plastic of choice for cup/tub containment of yogurt, cottage cheese, and so forth.

To obtain oxygen barrier, two plastic resins, polyvinylidene chloride (PVDC) and ethylene vinyl

alcohol (EVOH) are employed commercially. PVDC is the older of the two and has excellent oxygen, water vapor, fat, and flavor resistance, but is relatively difficult to fabricate. Much PVDC is used in emulsion coating form on film to achieve barrier in film used for cured cheese. EVOH is a better oxygen barrier material and is easier to fabricate, but is very sensitive to moisture. The economics of both high oxygen barrier materials dictate that they be combined with other less expensive structural plastic resins. Thus, EVOH is often coextruded (i.e., forced in parallel with another plastic through a common die) with polypropylene to produce films, sheets, or coatings. Thus, the EVOH is protected from environmental or product moisture in these applications. In addition to its involvement in "bucket" style cans, EVOH is also an increasingly important material to protect food and beverage contents from flavor interaction with packaging materials. With many food and beverage contents now being held for prolonged periods up to a year at ambient temperature, the probability of adverse product plastic interactions, largely flavor changes, is relatively high. Consequently, an intermediate high flavor barrier material such as EVOH serves to minimize such interactions in packages such as those for chilled juices.

Packaging Operations

No such entity as comprehensive packaging exists in a single organization even though comprehensiveness is indispensable to effective and functional packaging. All packaging is divided into a large number of individual units selected from a broad array of offerings to permit the dairy packager to select according to product, distribution, marketing, and so forth, need, or desire. These operations may be defined in tier or horizontal form. Examples include the suppliers of raw materials, converters, or those who convert these raw materials into useful packages, suppliers of machinery, designers, and so forth.

Raw Material Suppliers

Raw material suppliers include organizations which obtain basic materials from the ground or the oceans and transform them in large quantities into materials that may later be converted into packaging. Among such basic material organizations which generally do not supply dairies are aluminum refiners, steel mills, paper and paperboard mills, and petrochemical companies. Such companies deliver materials such as

coils of metal sheet, rolls of paperboard, rail car loads of plastic resin, and so forth, to converters. The principal exception to this is glass whose nature fosters the on site integration of raw material and converting into bottles and cans.

Among the packaging material suppliers of interest to dairy product packagers currently are in the area of paperboard, International Paper; Stone Smurfit and Weyerhaeuser, all of which manufacture virgin paperboard used in gable top cartons and or corrugated fiberboard cases. Basic aluminum suppliers include Alcan and Alcoa. Plastics suppliers include DuPont, Dow, and ExxonMobil and BP Chemical.

Converters

Converters are organizations which supply useful packaging to dairies packagers. Such organizations acquire commodity-type raw materials from their own suppliers and process and combine them to produce flexible films, sheets, cups, cans, tubs, bottles, cartons, cases, and so forth. Among the operations provided by converters are printing, cutting, molding, lamination, adhesion, cup formation, nesting, slitting, and coating. Quantities involved are generally much smaller than those offered by their suppliers, and sufficient for their dairy and food users.

Converters tending to focus on the dairy industry include International Paper; and Tetra Pak for gable top paperboard lamination cartons. Corrugated fiberboard case manufacturers include International Paper; Stone Smurfit Rock Tenn, and several hundred others. Ice cream carton manufacturers are headed by Sealright as the major supplier for bulk ice cream containers.

Metal can makers include Rexam and Crown Cork and Seal. Glass bottle manufacturers include Owens Illinois. Flexible packaging converters include Printpack and Curwood as well as several-hundred smaller firms. Many dairies extrusion blow mold their own HDPE milk bottles. Merchant plastic bottle blow molders include Consolidated Bottle Injection blow molders such as for polyester bottles include Constar and Ball, as well as many smaller companies and some self-manufacture. Cup and tub molders include Sweetheart and Solo (particularly for polyester cups). These are intended only to reflect a few of the wide array of suppliers which are available to dairy packagers to provide their package material needs. In almost every instance, there are many, but in no case can a single supplier provide a complete range of

all packaging materials which a dairy packager will require.

Packagers

Packagers are the dairy and food organizations which marry the packaging materials to the food and dairy products. Packagers employ machinery designed, engineered, and built by specialist firms.

Distributors

Distributors include the means to deliver the packaged dairy and food products to consumers. Distribution channels include warehouses, transportation, wholesalers, brokers, jobbers, retailers, and so forth. Retailers include grocery and food service outlets.

Suppliers in the Packaging Chain

In addition to these tiers, a range of suppliers provide goods and services to comprehend the requirements of comprehensive packaging. Graphic designers, for example, offer the artistic services converted by printers and molders into ready to display packages. The output of graphic designers is intended to comply with regulatory requirements as well as to meet the desires of marketing managers for retail communication.

Packaging Equipment

Not the least important of providers are the packaging equipment manufacturers, agents, and importers. Of some importance to dairy packagers are Evergreen Elopak, and Nimco for gable top paperboard cartoning. Tetra Pak now supplies not only its traditional aseptic packaging equipment but also gable top cartoning machinery. SIGCombibloc also manufactures aseptic cartoning systems. Among the aseptic plastic cup equipment suppliers are Bosch, Hassia, and Hamba, all European origin. Plastic bottle filling equipment is supplied by US Bottlers, and so forth. Cup filler may be obtained from Autoprod, Holmatic, and Sealright. Suppliers for flexible packaging equipment for products such as cheese include Hayssen, Multivac, Harpak, and CFS. Secondary packaging equipment suppliers include MeadWestvaco (MWV) and Graphic Packaging. Suppliers of machinery for distribution packaging include ABC, Salwasser, and Pearson.

Packaging Development

The development of packaging is a sequence that involves a broad range of disciplines and professionals who interact in overlapping order to finally deliver packages to the consumer. The most important consideration in developing packaging is product safety, with the interaction of packaging and contained food or dairy product being one element, and the interaction of processing and the package another. There must be no interactions that might extract desirable constituents from the contained food or dairy contents or introduce contaminants from the outside. Thus, the direct contact of post-consumer recycled package materials with their unknown genesis is rejected, unless the recycled materials may be totally transformed back to original form as with aluminum, steel, or glass. In general, paperboard and plastic materials may not be purified sufficiently economically.

No processing operation such as heating can compromise the safety as, for example, retort processing or ESL packaging which could conceivably disrupt heat seals and permit the entry of microorganisms.

The above are axiomatic in selection of package materials.

The next fundamental in the development of packaging is technical function. If the package cannot contain and protect the product in its normal and expected distribution channels, it has not fulfilled its basic objective. Thus, the initial step in packaging development is engineering to insure technical functionality. Variables such as moisture protection, seal integrity, protection against the entry of oxygen or microorganisms, resistance to heat or cold or both in sequence or randomly, product/package flavor interaction, and so forth, are specified and measured versus the ability of the package to meet the necessary and the desired criteria. Subsequently, the ability of the finished package in its totality to withstand the distribution environment using such measures as impact, vibration, and compression resistance may be predicted and measured.

In some situations, the effect of environmental variables may be predicted by mathematical models knowing the end objective in terms of first desired shelf life under specific temperatures, and second, the effect of the variable on that shelf life. A typical example might be a cheese product in refrigerated distribution which would have a target shelf life of 40 days. The model would input a variable such as no more than 1% moisture loss through the packaging material in that 40 days at 40°F as the key factor

to maintain quality. The model can then predict the gauges of the package material options that might be applied in the particular packaging structure being considered. Of course, mathematical models in shelf life prediction today are only guidelines, and so actual laboratory and/or field testing is required to verify the test results.

Distribution resistance may be mathematically predicted with fairly good accuracy, but actual laboratory testing is needed in almost every instance. In many instances, the use of actual real distribution is employed although, of course, the test variables of a single truck ride are such that the results are often misleading. Nevertheless, many packaging developers use actual truck shipments as a testing protocol in lieu of controlled vibration and drop testers.

Most of the time, the packaging development sequence is to extrapolate from known packages of similar products such as, for example, if the product is a flavor variation of an existing commercial product, relatively little testing is necessary, but some must be performed to ascertain the effects of such differences as flavor interactions.

Although laboratory or hand-made package samples are satisfactory for initial evaluations, it is necessary to conduct tests on real production samples since these invariably differ significantly from the pristine prototypes. When actual production line samples are not immediately available, the closer the samples are to machine-made, the better for real-life prediction.

While functionality testing, and its associated reengineering of the package structure is underway, the marketing inputs are incorporated into the package. These include the graphic requirements, both legal and desired, structural features such as pour spouts, reclosure tabs, tamper-evident/tamper-resistant devices, and so forth. Whenever a structural change is made, the resulting package should be re-evaluated, but, in practice, this step is not always performed in the haste of meeting marketing schedules. Alternatively, a more robust-than-needed package is employed on the premise that the conservatism will assure protection.

Today, graphic design can be and is being performed directly on computers, and so rapid action is quite feasible. In a large project, it is essential that the package design including all its structural features undergo both consumer and retail display testing. Too much investment has been made in the package to avoid this key step, although, of course, many dairy product companies may overlook it. A

host of consumer and marketing research firm conduct such tests ranging from focus groups to actual in-store displays or in-home testing. None is perfect and comprehensive, although, of course, each purveyor of a test procedure believes and claims that his or hers is the ideal measuring tool. The most important marketing design test must be the simulation of in-store display, that is, perception of the package by consumers in the normal shopping environment as in a mass display among other similar and competitive products. Yet another necessary test is how consumers actually use the package to deliver the product to themselves to ascertain any consumer perceived flaws or areas in which the package design may be improved.

During the development of the package, it is necessary to select the equipment on which the package is to be made to insure that the product, packaging material, and machine are compatible. Machine reengineering is not only feasible, it is to be encouraged prior to completion of development. Package and equipment development should be a totally integrated effort and should be continuous from the inception of any dairy product packaging development project. As much as possible, off-the-shelf equipment should be used since custom equipment development requires heavy investment. Standard equipment can be modified to accommodate special requirements of product and package.

Not the final step in initial development is secondary or tertiary or distribution packaging. Here, both the package and the equipment must be developed and selected or modified for the unitization and containment of primary packages for distribution in the specific channels.

Throughout the packaging development process, it is necessary to quantify economics of the package including the cost of the package materials and structures, equipment, labor, utilities, and so forth. Each should be developed in a total packaging systems context to insure that the economics are not dictated solely by the purchase price of the package materials, a variable that can be highly misleading in the context of the total distribution and marketing objectives.

All packaging development must include continuous monitoring and refinement to insure that some environmental variable has not changed, or that there has not been a change in the product, or that some improvement in package materials has not introduced the possibility of effecting a change to better the performance or the economics, or both.

Resources Available for Packaging Development and Implementation

In addition to the direct sources indicated above for providing the hardware and software are many other resources that are not always immediately visible. As indicated above, graphic designers are valued suppliers since, unlike mainstream advertising agency or free lance artists, they are experienced in packaging design including the peculiar nature of shelf display and the vagaries of packaging material converter printing.

Consulting firm (such as Packaging/Brody, Inc. and its affiliate Packaging and Technology Integrated Solutions—PTIS) deliver a variety of accurate insights into the technologies of packaging. If desired they can engineer and test the package structures. Most consulting organizations, if they are indeed organizations and not single persons, offer advice based on information not gleaned from experience but rather exposure. Dairy product packagers seeking insights from consultants are urged to study their dossiers carefully to determine that their counsel is really sound information and not merely superficial bits of little or no real value or supplier-driven recommendations. It is also important to ascertain that the counsel is coming from the professional with whom the communication is made; many larger consultancies often delegate the actual consulting to persons who are either junior and thus inexperienced or who are not busy, and so the inputs contain little relevant substance; the assigned person(s) have been learning about the topic during the consultancy assignment.

Many packaging journals are published in both the United States and other parts of the world. Each is distinctive in its coverage of packaging subject material, but almost all share one characteristic: they are assembled and edited by journalists for maximum reader interest. Despite the reporting and investigative research behind the published pieces, they often lack the critical insights that a packaging professional would infuse. Further, there is little follow-up to ascertain progress on developments. Thus, packaging journals provide a highlighting service that can represent an education for novices, and a stimulus for those who function in packaging on an every day basis, but for in-depth information, there is no outstanding periodical today. Nevertheless, the roles of *Packaging Digest* with its large sprawling personalized articles, *Packaging World* and *Food and Drug Packaging* cannot be minimized: all offer good timely information and are must reading for dairy packaging

professionals. The one peer review packaging journal, *Packaging Technology and Science*, offers critical reports on a variety of research and occasional excellent review articles on contemporary packaging topics.

Nonpackaging periodicals such as *Dairy Field* cover packaging with rewritten press releases, reporting or, occasionally, professionally prepared pieces. Unfortunately, such journals do not provide regular information on packaging and cannot be depended upon as sources. On the other hand, when there is coverage, the information on the specific application is usually quite good. *Food Technology* contains a monthly analytical column on food packaging prepared by this author and is often considered a good reference for topical information.

A number of books on packaging have been published, including some by this author. The books are usually general texts and contain only brief or passing references to dairy packaging per se. To date, to our knowledge, no definitive text on dairy products packaging has been written and published. Dairy products texts often contain single chapters on packaging like this one which is necessarily sketchy since such a broad field must be covered in such a short space.

Professional and trade associations both publish information on packaging and sponsor meetings and conferences on the subject. Those by the United States' main professional society, Institute of Packaging Professionals, generally emphasize more general packaging topics rather than focusing on specific of a particular group such as dairy. The reverse is true for dairy associations which tend to focus on the mainstream of dairy products rather than on packaging for dairy products. On the other hand, when a professional group covers a dairy packaging subject, it tends to be quite good.

Both professional and trade associations, as well as for-profit companies organize and produce exhibitions and conferences on packaging and on dairy products. There has not yet been an American dairy products packaging exhibition, although in Europe, an excellent dairy packaging exposition has been presented from time to time. The major world packaging exposition is Interpack held every 3 years in Germany, but generally absent of much direct dairy packaging. In the United States, the major packaging exhibition is Packaging Machinery Manufacturers Association's (PMMI) Pack Expo held in alternate years in Chicago and Las Vegas, but also suffering, in this context, from a paucity of dairy packaging. Regardless, packaging professionals involved in dairy packaging

have much to gain from alert attendance at major packaging exhibitions which usually present much that is innovative and indirectly applicable to dairy packaging. Fourteen American and Canadian universities offer degree programs in packaging. The largest such program is at Michigan State University in its School of Packaging. Both Michigan State University and Clemson University affiliate their packaging programs with their foods science and technology curricula. Among the other universities offering packaging are University of Missouri (Rolla); Rutgers, the State University of New Jersey; Rochester Institute of Technology; San Jose State University; and Guelph University in Canada. Generally, universities offering curricula in food science and technology have a single course in food packaging presented by a faculty member who may or may not have ever had a packaging course or any experience in packaging. Dairy curricula may sometimes offer a course in packaging from a faculty member. A few universities have research programs dealing with dairy packaging, the most prominent of which is North Carolina State University with an aseptic packaging center.

A very limited number of federal and state government agencies conduct research in packaging, with the FDA being the most prominent among these focusing, as might be expected, on safety aspects. Offshore, however, government and quasi-government agencies, perform both basic and applied research on packaging. Among these are Campden Chorleywood Food & Drink Research Association in England, the Detmold Institute in Germany, and SIK in Sweden.

All these groups represent resources that should be employed in comprehending the totality of packaging as it applies to dairy packaging issues.

Interactions of Product and Packaging

As has been indicated above, it is axiomatic that no significant interaction takes place between the contained product and the package material. This is particularly important in considering the possibility of any potentially toxic materials being extracted from the packaging materials into the contained product, an event specifically prohibited by law and regulation. From a business perspective, any interaction that perceptibly alters the quality of the contained product is highly undesirable.

While the notion of extraction is easy to understand, it is also necessary to grasp the idea that extraction can occur not only in what might be regarded

as normal contact, but also under unusual conditions. For example, migration of packaging material constituents can occur in dairy product distribution, which may take place at ambient, chilled, or frozen conditions. Migration rates vary considerably under the three different temperature conditions, with ambient temperature rates generally higher in accordance with Arrhenius laws. But, if the product is placed in contact with the interior packaging material at an elevated temperature during some processing or consumer preparation time, migration can be greatly accelerated, thus leading to brief, but nevertheless significant component migration. This situation has become evident in the case of microwave susceptors whose migration patterns in original processing, packaging, and distribution demonstrated benign activity. When the susceptors perform their function of surface heating, however, brief very high temperature periods occur during which new chemical entities are formed which may migrate during the interval from the packaging material into the food. Although this specific situation has not proved to present any public health problems, it has alerted both official and food packagers to the possibility and the potential consequences. Thus, all package material testing protocols now dictate evaluation under normal conditions of use.

The microwave susceptor case also highlights another effect that was initially demonstrated with retort pouches many years ago: indirect migration. The term *indirect* is used in regulatory contexts to indicate a component that is not intentionally introduced into a food or dairy product, but enters from a secondary source such as the surrounding packaging. In this context, however, indirect means that the component comes not from the package material in direct and intimate contact with the product, but rather from a layer that is remote from contact, for example, an adhesive or an outer ply. In this situation, the migrant not only leaves its own substrate, it also migrates across other packaging components to the surface of the inner layer and potential contact with the contained food or dairy product.

Contact is not necessary since the migrant might evaporate or sublime into the interior package environment and then be borne to the food surface for potential interaction. As indicated above, these actions are accelerated at elevated temperatures, even for brief time exposures.

One variable that was not always considered was that for most of the history of packaged dairy products, contact between plastic and contents was

usually brief and at relatively low temperatures, thus minimizing or even hiding any adverse interactions. With the development of aseptic packaging systems and hot fill into plastic packaging, product/package contact time at ambient temperatures extended to weeks, months, and even, occasionally, years. Under these circumstances, measurable interactions can take place, with some caution required to insure against harmful migration. Some of the interior packaging materials such as polyethylene are not inert to organics, and so long-term exposure can and does result in undesirable interactions. Since no known package material contains or transmutes to components that might be harmful in consumption, and this effect is very carefully monitored by plastic resin suppliers, the probability of a public health problem is almost absent. On the other hand, interactions that can alter product quality can occur, and even if they are not harmful to consumers, they can cause eventual declines in sales. Thus, all packaging should be tested to insure that under the total conditions of processing, packaging, and distribution, no measurable interaction of product and packaging occurs. If such interactions are identified whatever is extracted must be determined as safe under regulations. In recent years, even regulatory assent may not be acceptable to some consumer groups who are persuaded that any contact with plastic must be harmful.

The reverse of entry of undesirable materials is the removal of desirable constituents, another of the issues of employing plastics in proximity with the dairy product contents. Scalping or loss of product components to the contact package materials has been a phenomenon known for many years, but largely overlooked since only infrequently was there any prolonged contact time of plastic and liquid or fluid product at ambient temperature. Since the advent of aseptic packaging, however, long time intimate contact was initiated and conditions were established for the plastic material to remove desirable product compounds. These have been largely lipophilic compounds that dissolve in and diffuse through polyethylene, but also include volatiles which normally contribute to the desirable flavor attributes. Many juices are subject to "scalping," an event that has led to the replacement or modification of the interior plastic heat sealants with more inert plastics such as polyester, nylon, or even EVOH.

Measurable losses may be found in long-term refrigerated distribution. For example, during the 60 + day chilled life of juices in gable top polyethylene-coated paperboard cartons, the desirable flavor

constituents are scalped sufficient to be detectable by consumers. To overcome this serious problem, chilled juice packers are now specifying fl vor barrier plastics on the interiors of their cartons. No reports of scalping of desirable fl vor constituents of dairy products by polyolefin have been noted, but lipid-soluble volatiles might be expected to be found in interior heat sealant layers which could lead to fl vor deteriorations over time. Dairy product packagers should be alert for this possibility in developing packaging for their products.

Yet another interaction that should be of concern is the change in package material properties over time or in contact with either product contents or the environment. For example, paperboard loses most of its physical strength when exposed to water or water vapor. Consequently, protecting the paperboard itself is essential to the protection of the product. Wet strength paperboard has been a standard for years, but this is only a small temporary expedient. Closing all raw edges and seals is another more expensive, but significant step almost always employed for long-term distribution such as for aseptic and ESL packages.

Because of their hydrophilic properties, nylon gas barriers of cured cheese packaging may be altered by the inevitable presence of moisture and must be accounted for in developing packaging for any dairy products. The situation with the newer oxygen barrier, EVOH is even more severe, since as much as 75% of the gas barrier properties can be lost because of the moisture sensitivity of this plastic. Even under these circumstances, these two moisture sensitive plastics are commercially employed for dairy product packaging because even after property losses, they represent gas barrier superior to the alternatives.

These recitals on problems with plastic packaging materials hint that possibly avoidance of plastic would be a desirable alternative—especially in the face of vocal environmental advocacy. Given that plastic materials are imperfect, in total, they generally represent a better alternative than almost all the others. Attempting, for example, to package milk in uncoated paperboard which has no liquid barrier, or in glass which would be both expensive and hazardous to consumers would be folly particularly in these litigious times. Further, the cleaning of glass in reusable packaging operations, is not devoid of problems relative to energy, breakage, and the persistence of residual cleaning compounds. Metal cans would be an alternative, but metal must be plastic coated in the interior to protect the metal, and so this alternative leads to almost all the problems associated

with plastic in contact with product so widely publicized.

It is better to employ the packaging with the best combination of properties knowing in advance issues that might be encountered, and to account for them rather than to use a sub-optimum material or structure. If plastics appear to present serious problems in this context, consider the alternatives which, in reality could present even more serious major problems.

Yet another issue that should be addressed is the employment of package materials and structures obtained from offshore sources. Although there have been many headline stories of Asian origin contaminated foods and toys, little publicity has been given to a more insidious variant: defective imported package materials and structures. Most package materials from offshore locations are probably acceptable since they are used broadly on a commercial scale. Some, however, have been demonstrated to be deficient and even possibly hazardous because, for example, of residual organic solvents. Asian manufacturers are not governed by United States' Environmental Protection Agency regulations and so may use volatile organic solvents without proper safeguards. Residuals are often difficult to detect and may pass perfunctory quality assurance procedures with little notice until they reach the retail marketplace, with devastating results. Thus, the seemingly lower cost of package material acquisition from offshore sites may turn out to be extremely expensive. Caution is urged when dealing with low-priced offshore package materials and structures and their representatives.

The Package in Product Distribution

Among the many functions of packaging are to protect the contents from physical abuse such as vibration, impact, compression, and so forth, and to fit onto pallets and into truck bodies. The notion of delivering dairy products one package at a time is obviously preposterous. Therefore, primary packages such as bottles or cartons should be unitized into groups that are more easily moved en masse. In the distribution cycle, all packaging including the primary packages must be protected throughout the entire distribution cycle, including warehousing, transport, docking, retailer, and so forth. The primary package itself must be able to withstand retail display, handling by the consumer and in home or food service operations. Because the primary package is the major barrier against the environment, it is necessary to engineer it to remain intact in the entire distribution cycle. It must withstand

physical stresses such as would be encountered in distribution such as impact, vibration, abrasion, turning corners, occasional compression, and, in dairy plants, water and water vapor and in retail display, water vapor. Subsequent to the packaging lines, the primary package is multipacked, sometimes under compression, sometimes by dropping, but in any case, to be further protected by some outer unitizer. The next outer package is often a corrugated fiberboard case which has been engineered to resist modest impacts, compression, and drops. Unfortunately, corrugated fiberboard cases are susceptible to moisture and water, and thus lose their protective properties rapidly as a result of exposure. This vulnerability must be accounted for when employing corrugated fiberboard as a distribution package.

Alternatives to the corrugated fiberboard case include corrugated fiberboard trays or pads combined with plastic (usually LDPE or a variation) shrink film capable of tightly binding primary packages into a single unit that is stronger than the individual primary packages because of the "cellular" construction. Plastic shrink film is also a good moisture barrier and so helps to protect interior paperboard from the inevitable moisture of dairy product distribution environments. The small amount of heat required to shrink the film around the unitized primary packages is so inconsequential that even ice cream packages are readily unitized and held together by heat shrunk plastic film.

Many dairy products are distributed in returnable plastic crates or cases. These injection molded HDPE or polypropylene co-polymer units are engineered to cradle and contain numbers of primary packages to protect them from virtually any physical abuse. Often, the individual primary packages are in cells within the plastic crate to prevent the primary packages from any contact with each other and thus eliminate surface abrading which can damage glass bottles and even paperboard cartons. When the dairy's distribution system permits return of the relatively bulky and expensive returnable plastic case, it makes physical and economic sense to employ such distribution packaging. The initial capital investment is high but the total system cost over time, when the infrastructure is available and in place is well below that of purchasing individual disposable distribution packages.

Distribution stresses and the protection afforded with excellent predictability. These methods are more often employed by packaging engineers associated with high price hardware items, but the test bed

and computer techniques may be readily applied to distribution packaging for dairy products. In the system, test packages are subjected to known stress inputs such as vibration or impact, and the point of failure is quantified. Knowing the properties of alternative distribution packaging such as corrugated fiberboard of a specific edge crush test and dimension, or an internal egg-crate type structure, computer modeling can predict the distribution performance. In this manner, the minimum distribution packaging required to protect the primary packaging may be derived by computation rather than by empirical methods. Nevertheless, it is advisable to conduct actual test shipments to verify the theoretical results and reduce the tedious, and often very inaccurate trial and error methods that previously were the hallmark of distribution packaging selection.

Graphic Design and Assessment

Graphic design is the development of the external appearance of the packaging to comply with regulations and to meet marketing desires that are, hopefully dictated by consumer and retailer needs and perceived needs. Good graphic designers also incorporate structural features that are not incompatible with the protection requirements of the product, but are compatible with retail display or consumer use, for example, dispensing spouts. Good graphic design is performed to insure that the package appearance in retail display has visual impact in mass among an array of other competitive packages. Designs may appear excellent in isolation, but in mass display, they might be lost. When media advertising is used, it is necessary to insure that the package appearance is attractive in photography or on television as the case may be.

Graphic design is best managed from a marketing department since this is the group that is most influenced by the shelf appearance of the package. It is important, however, that the dairy packaging development professional be actively involved in to insure that the technical requirements are not compromised for the sake of appearance.

Graphic design today should be performed by packaging design professionals. The use of freelance or advertising agency artists with no experience in packaging design is to be discouraged. It is even better to employ professionals with experience in dairy products packaging. Today, many, if not most, graphic designers are able to design on graphic computers, permitting marketing managers to see

design variations immediately. Three-dimensional views may be depicted on the two-dimensional video display screen, and hard copy versions. Mass displays can be represented on video display screens. Almost instant color copies may be wrapped around physical structures to enable marketing managers to actually see and touch three-dimensional samples immediately. While permitting "instant" packaging design, computer graphics has also generated multiple variations for evaluation. Computer graphic capabilities are so sophisticated today that the camera ready art for printing plate manufacture may also be generated and transmitted by computer.

With design being so critical for market acceptance, personal opinion by marketing managers or graphic design managers is not a good method of selecting the optimum design. Objective evaluation of design is nearly as important as evaluation as is consumer testing of the product. If the consumer does not try the product or cannot find the product, it is of little value to the dairy. Many different techniques for packaging (graphic) evaluation are offered, none of which is generally accepted, but each of which has its own advocates. The most common probably is focus group in which a small group of target consumers examines and discusses the totality under guidance of a moderator. Among the more intriguing are measurement of eye movement, time required to recognize the package on a darkened screen, and measurement of brain waves exposure to the design. Perhaps the best method is placement of the package in a mass display in a test store environment followed by measurement of sales and follow up with a selected sample of purchasers to ascertain their reasons for their decision.

Economics of Packaging

Contrary to general belief, with infrequent exceptions, packaging does not cost more than the product contained. Generally, packaging costs represent considerably less than 10% of the price of the food or dairy product on the retail shelf.

Not long ago packaging costs were generally computed solely by the cost of the primary packaging materials purchase price. With education, however, packaging purchasing and marketing managers can measure packaging costs by adding all relevant variables and allocating all fixed costs including capital expenditures for equipment. Thus, the economics of packaging include such costs as those for the acquisition of the primary packaging materials plus the

secondary and distribution packaging materials plus the labels, adhesives, coding inks, and so forth, that is, the adjuncts, plus the labor, plus the utilities, and so forth. In addition, allocation of fixed plant costs such as supervision and maintenance, floor space, and so forth, are included. Just as important in determination of economics of packaging is the machinery which invariably has a high initial cost, and must be evaluated for output, output speed, efficiency, scrap losses, both for packaging materials and product, down time, and ability to link efficiently with both downstream and upstream packaging equipment.

Only after examining all facets of packaging costs can the true economics of packaging be accurately evaluated. Soon, the days of judging packaging costs on the basis of number of colors on the label, such as was and perhaps still is being done for the "no frills" levels of packaged food and dairy products will be ended. There is much more to packaging economics than number of colors printed which is usually a trivial contributor to the total economics.

Regulation

Beyond the regulatory issues relating to safety of packaging materials and the contained products are the regulations governing on-package information, that is, the so-called labeling declarations. As should be well-known to every dairy technologist, a host of federal, state, and local agencies have some manner of label jurisdiction over dairy product packaging.

The most important of these, of course, is Food and Drug Administration (FDA) whose authority takes precedence. Were the products meat, U.S. Department of Agriculture (USDA) would have jurisdiction, taking their lead from FDA especially in package properties. A considerable amount of authority, usually unexercised, rests with the Federal Trade Commission which has power to regulate relationship of packaging information to advertising and in addition to those with legal authority are the quasi-legal groups and trade "regulations" which stipulate packaging information requirements. For example, the Railroad Board stipulates the mandatory labeling relating to board strength on the corrugated shipping cases. Supermarkets dictate the presence of a machine readable Universal Product Code (UPC) on primary packages.

FDA regulations prescribe four major information items on food and dairy product packages including the generic identity of the contents, net weight, source of the product, a list of ingredients in

descending order or weight or volume importance and a table of nutritional information. In addition, if the manufacturer/marketer makes any claim on the package or in advertising or promotion, regarding the nutritional value or health benefit of the product, this must fall within a specific set of guidelines.

FDA has established a set of rules for Good Manufacturing Practices, many of which are aimed at insuring that the packaging is safe, not only from a content standpoint, but also from a processing and containment perspective. Specific rules for handling low-acid foods, and many dairy products certainly fall into that category, have been issued. These rules might be regarded as the common sense rules of operating a food or dairy processing or packaging line, formalized as a regulation. For example, anyone operating a retort must be trained in retort operation. Complete records must be kept for all low-acid retort operations. Closures for cans and glass jars for retorted low-acid foods are specified. Regulations for aseptic packaging especially with regard to thermal processing of contents, sterilization of packaging materials, and seal integrity are stipulated, with provision made for application to FDA if the system has not been used previously in commercial practice. FDA also requires that any organization packaging and processing low-acid foods for ambient temperature distribution submit its process to FDA prior to initiating operations so that FDA can ascertain that the persons and equipment and operations are qualified to function. This is one of the few areas in which the FDA requires prior approval for an operation.

The several highly publicized incidents of tampering with over-the-counter drugs and a few foods that occurred in the 1980s triggered a number of laws and regulations stipulating tamper evidence—tamper resistance for these drug products. Simultaneously, many food and dairy processors and packagers implemented tamper evident—tamper resistant package features both to deter criminal intent and to deter innocent in-store taste testing and content contamination. The regulations apply only to the proprietary drugs, and so food and dairy processor/packagers are not required to follow the specific guidelines of the FDA regulations. Nevertheless, almost all food and dairy packagers that have incorporated tamper evident—tamper resistant features are, in effect using the regulatory guidelines. These guidelines specify a number of devices which are regarded as being generally effective and the presence of a printed instruction to signal to the consumer the absence of the device, or a tampered package.

In general, government regulations regarding processing and packaging of food and dairy products are quite good and make very good sense to all food and dairy packaging technologists. There is little onerous about any of the regulations since they are merely reiterations of good technical and commercial practices designed for delivery of safe products in packages that communicate accurate information.

Packaging and the Environment

The most widely discussed and debated aspect of packaging today is and has been the environmental impact of the solid waste generated from packaging. The issue has generated more laws, regulations, consumer actions and reaction, and media discussion than the combined total of all other issues, even safety, related to dairy product packaging for the past decade.

According to the environmentalists fostering this issue, packaging is the major component of the municipal solid waste stream, and should be eliminated or made of nothing but materials that have been recycled from the solid waste from consumers' homes or at least in a wholly sustainable manner. If not, the earth's energy and physical resources will be depleted and land fill will overflow with this solid waste and contaminate the soil, ground water, and air. As a result of these cries which have been largely unrefuted by the food industry as a unified force, hundreds of laws and regulations have been passed restricting food and dairy packaging, or at the very least, dictating household separation of packaging solid waste and "curbside" placement for "recycling" pickup. Just as significantly, thousands of laws and regulations have been proposed to limit packaging, including stipulating minimum contents of "post-consumer" solid waste to be incorporated into the packaging materials. In extreme instances, packages have been banned, as in the State of Maine where the paper-board/plastic/aluminum foil aseptic brick/block pack was largely banned on the grounds that it is "not recyclable," an action that was ultimately rescinded.

At the moment of this writing, the most visible movement is sustainability, design of the entire packaging chain to compensate the earth for use of energy—of manufacture, transport, use and eventual regeneration, and physical entities such as trees, ore, and especially petroleum consumed in converting to plastics. Offshoots include the trends toward banning of plastics such as plastic grocery bags and water bottles. Pressure has been building to markedly

reducing if not totally banning plastic packaging. Corollary to this movement has been a growing notion of acquiring all food from local producers on the grounds that such actions will reduce processing energy, fuel consumption from farm to plate, and package materials mass.

The actual facts refute almost all the claims regarding the role of packaging in the solid waste stream, and the chronology of the environmentalists' movement in this regard reflect abrupt turns to reflect the reactions to initial misinformation that precipitated most of the laws and actions. For example, at the outset, most environmentalist groups cited "biodegradable" packaging as the best answer to the problem of solid waste, but after it was clearly demonstrated that biodegradation does not occur in reasonable time within properly constituted sanitary landfills biodegradability was virtually erased as a viable alternative. When recycling was demanded on the basis that no package materials were being recycled, the food and packaging industries responded with valid data demonstrating that rather large percentages of spent packaging materials were already being recycled. For example, well over one-half of all paperboard and folding carton materials, 20% of glass, and 60+ percent of aluminum cans are annually recycled. When the surprised environmental groups learned that these data referred to materials generated largely at packaging material converting plants they shifted their targets to "post-consumer" package materials. It seemed that just holistically viewing the solid waste system on a common sense economic basis was insufficient. Further, many post-consumer packages could not be safely recycled into safe packaging because of the unknown history of the packaging, in contrast to the relatively safe sourcing of industrial packaging waste.

In actual fact, the municipal solid waste stream consists of about 150 million tons, which, by any measure, is formidable. Less than 30% of the municipal solid waste stream is packaging, a proportion that has been declining, even as the rate of growth of the stream has been declining. By FDA regulation, the incorporation of post-consumer waste paper into package materials that might contact food or dairy products places consumers at risk from the potential hazards of unknown contaminants that cannot be removed except at great cost.

Properties of recycled paperboard are quite different from those of virgin and the two cannot be used interchangeably in all applications.

When they are exchanged, the properties are such that additional recycled paperboard is required to

compensate for the physical property losses, thus generating a higher quantity of solid waste and forcing increased use of energy to transport the new "recycled" packaging. This is not to say that recycled paperboard packaging cannot be used; it has been very successfully used for decades in secondary packaging, and will continue to be used in such applications. Without imposed laws, the paper and paperboard industries have functioned well, using economic laws.

The cost of returning used glass packages to the rapidly decreasing number of glass bottle plants in the United States is too high for economic justification; nevertheless, many municipalities are trying to do that. In our lifetimes, there will be no economic driving force for spent glass return, with one probable result being that the decline of glass as a packaging material will be accelerated.

Because the price of aluminum is so high and because aluminum may be safely and economically recycled, aluminum can recycling has been commercial practice for more than four decades, or since the aluminum can captured a lead in beer and carbonated beverage packaging. A recycling infrastructure has been in position and functioning well. Aluminum can recycling often is cited as a model of how recycling can be effected, but without mention that the system was in position long before environmentalists activists were active, and because of economic driving forces that could be accommodated without endangering the public health. A drawback to the aluminum recycling program is the absence of aluminum foil from the stream. Aluminum foil is generally laminated to plastic and/or paper and is difficult to separate economically. The visibility of this relatively trivial amount of aluminum in the municipal solid waste stream has led to disproportionate efforts to "cope" with the problem.

Plastics have been the particular target of environmental agitation and regulation on grounds that plastic never degrades in landfill and that is an unnecessary expenditure of our limited planetary energy resources. Consumer (and politician, food and dairy technologist, marketer, journalist, and so forth) perception is that plastics constitute over half of the total packaging solid waste stream. The reality is that plastics constitute about 10% of the weight of packaging, the smallest fraction of all materials. The density of plastic packaging is low so that the space volume is high. Using the volume criterion, plastic packaging constitutes 20–25% of packaging solid waste volume. This argument is spurious since almost all packaging waste is compressed under high pressure to flatten everything.

Further, no single plastic material is employed for all packaging. About six plastic materials, each of which has different properties, are used for packaging, sometimes together. The variation in use dictates that no single plastic material constitutes more than perhaps 3% of the packaging solid waste stream. This small weight of single plastic material in the packaging solid waste stream retards effective recycling efforts for any single material. Nevertheless, efforts are underway to develop infrastructures, technologies, and markets for recovering and using spent plastic packaging materials.

One might argue that these efforts are in direct response to the pressure from environmentalist advocates, which might be regarded as good for a sustainable environment, or might also be regarded as an artificial response which is merely symbolic and of little meaningful value. One might argue that the distorted misinformation communicated by sustainability supporters has generated an unhealthy emphasis on a package material that is in reality beneficial to the environment (if that argument can ever be made for anything) with the ultimate result being that the consumer is paying more for everything because the cost of "recycling" plastics increases the system price. For example, today's most publicized biomass-based plastic costs more than the petroleum-based plastic it is supposed to supplant. On a small base, increasing quantities of post-consumer plastics are being recycled into marketable products. Technologies are available to recover plastic from post-consumer solid waste streams, and although few are cost effective, many systems have been implemented.

Both the Environmental Protection Agency and responsible professional and trade organizations have developed a hierarchy of means of "coping" with packaging solid waste, with EPA also indicating that their recommendations deal with all of solid waste and not just the minority that is packaging. Their order is source reduction, recycling, incineration, sanitary land fill and degradability. Not included in their agenda is outright bans such as proposed so openly, or passed so readily by so many legislative and regulatory bodies.

Source Reduction. The reduction in the quantity of package materials used to contain food and dairy products is important. Source reduction is and has always been one of the primary activities of food and dairy packagers. Since these business entities generate sales and profit by delivering the best products at the lowest cost, reducing the cost of packaging by

rendering it more efficient is normal operating procedure.

Recycling. This category may be divided into reuse of packages directly for the same purposes such as returnable glass or plastic bottles, a procedure that involves caution relative to product safety; closed loop recycling which means reuse of the packaging material for the same application; and recycling of the spent materials into some useful but not necessarily similar application (which is often not packaging). Much of the commercial activity centers on recycling into some packaging application that is not the same as the original or into a nonpackaging application. Among the more advanced packaging material recycling efforts as of this writing are aluminum cans returned to produce aluminum cans, HDPE milk and detergent bottles into liquid detergent bottles, polyester carbonated beverage bottles into polyester carpet fiber and insulated jacket filling glass bottles into new glass bottles, and paperboard into recycled paperboard cartons or corrugated fiberboard fluted medium.

Incineration. When paper and plastic are incinerated in proper facilities, the energy generated can be used to produce heat or electricity, useful and cost-effective outlets. Well-engineered incinerators can burn waste efficiently with no air contamination and little residual ash. The ash represents significantly reduced solid waste volume from the input materials. Although the initial capital costs are high, financial returns can be very good from the sale of steam or electric power. Obstacles to waste to energy plants include consumer perception, particularly of the property values; dirt and air pollution; the high volume of truck traffic necessary to feed the input scrap; and the disposal of the ash which is perceived to be high in undesirable heavy metal elements. The "not in my backyard," "not in my term of office" syndromes dominate the development of effective waste to energy incinerators.

Degradation. Self-degradation was viewed by many in the environmentalist movement as the ideal answer to packaging solid waste. So called biodegradability would remove all packaging, particularly plastic, solid waste just as soon as the packaging had performed its protection function. Data from archeological studies of land fill indicate, however, that when land fill are intended for eventual use for building foundation or recreation area, a base that would degrade over time would be highly

undesirable. Self-degradation also, of course, interferes with recycling efforts. As a result of these findings, which arrived after the early efforts to change all plastic packaging into “biodegradable,” the concept has been largely discredited as an effective means of dealing with packaging solid waste. Yet another issue of degradable plastics is the unknown end products of self-degradation which could be toxic or even more destructive to the environment than the perceived adverse effects of packaging solid waste.

Nevertheless, efforts and investments are underway to develop and produce degradable packaging materials, with the term *degradable* meaning either biodegradable or photodegradable. Currently, major efforts are underway to market biomass-based materials which are capable of composting, another sustainable application.

Food and dairy interests are working diligently to minimize both the real and perceived effects of packaging on the solid waste stream. These efforts are sometimes interpreted as admission that a particular package or material is indeed at fault. Professionals experienced in food and dairy packaging are highly sensitive to the role of packaging in protecting the contents on behalf of the consuming public, and of the resultant relatively minimum contribution of packaging to solid waste. Regardless of the facts, the packaging community is working toward the resolution of the real problem, but attempting to employ only rational technical and economic means. And one hardly rhetorical question that emerges from all this discussion, after the food and dairy packaging issue has been resolved, what happens to the other 99% of waste generated in this and other countries?

PACKAGING SYSTEMS FOR DAIRY PRODUCTS

To this point, this chapter has addressed food and dairy packaging principles and not focused on dairy products packaging. This section deals with the specific applications and descriptions of some of the major systems in use or proposed for dairy products packaging.

PASTEURIZED FLUID MILK

Pasteurized fluid milk and derivatives generally are distributed under refrigeration and so are not expected to deliver long shelf lives.

Glass Bottles

The classical package for pasteurized fluid milk has been the returnable/reusable glass bottle which is cleaned after each use, refilled with the milk, and resealed with a reclosable but disposable closure. Returnable reusable glass bottles still are used occasionally in the United States, but are generally regarded as archaic even if they are advocated by environmentalist advocates.

Returnable Plastic Bottles

Returnable plastic bottles have been introduced into the fluid milk distribution system. Any returnable/reusable distribution system requires a distribution infrastructure that can recover the used containers and return them efficiently and economically. As of this writing, this system does not exist in the United States.

Plastic Pouches

For decades, flexible polyethylene film pouches have been used to contain fluid milk and analogues such as coffee lightener in bag-in-box configuration largely for food service applications. The “box” is corrugated fiberboard case for structural rigidity. Dispensing is through a device heat welded into the plastic film at the bottom. For aseptically packaged bag-in-box milk, an aseptic dispenser is available so that the contents do not become contaminated through multiple uses common for bag-in-box in food service size. Bag-in-box is prepared by employing premade/predevice affixed bags which are filled through the dispensing closure and then closed. For aseptic mode, the bag is presterilized by ionizing radiation. In either ESL or aseptic modes, the fill area is cleaned and/or sterilized prior to filling.

In Europe, Latin America, and Canada, consumer size pouches fabricated from medium-density or linear low-density polyethylene film are commonly used for fluid milk. A particular type of polyethylene is required to achieve an effective heat seal to insure against leakage either during filling or distribution. The strength of the resulting 1 liter or similar sized pouch is such that it resists impact from drops and from internal hydraulic action by content movement. The pouch is filled on a vertical form/fill/seal machine especially engineered for liquid filling. The filled pouch is intended to be inserted into a rigid plastic pitcher, cut open by the consumer and dispensed.

from using the opened pouch in a rigid plastic pitcher. This concept, however efficient of materials usage is, has not been accepted in the United States despite considerable marketing efforts over the 30+ years of its practical existence.

Tetrahedrons

Developed as the original structure by Tetra Pak in Sweden more than 55 years ago, the tetrahedral shape enjoyed a brief popularity for fluid milk and dairy product packaging because it employed less package material per unit volume contents than any other commercial package. The shape continues to be occasionally used in Europe and in North America for liquids, despite its awkward shape for inclusion in distribution packaging, and difficult of shelf display, in-home storage, opening, and dispensing. Tetrahedrons for pasteurized fluid milk containment are fabricated from roll stock polyethylene coated virgin paperboard, or if for ambient temperature shelf stable contents of a lamination of paperboard/polyethylene/aluminum foil. The package is filled and sealed on vertical form/fill/seal equipment on which the two transverse seals are at 90° angles to each other. The internal polyethylene coating serves as the heat sealant.

Plastic Bottles

In gallon and half-gallon sizes, extrusion blow molded HDPE bottles are the most popular package for fluid milk and its analogues. The weight per unit volume of fluid contents is the lowest of any packaging structure that can be opened, reclosed and comfortably dispensed. Quite often, HDPE bottles are blow molded off line in the dairy's back room. For short-term refrigerated distribution, HDPE is inert to flavor changes, and since little oxygen barrier is required for the short distribution time involved, the plastic's deficiency in this regard is of little consequence. On the other hand, HDPE is an excellent water and water vapor barrier and therefore is well suited to contain fluid dairy products. It is a low-cost and easy to fabricate plastic packaging material which provides a bottle that is tough and impact resistant. Bottles are filled at speeds of up to 100 bottles per minute on standard in-line or rotary turret liquid gravity filling equipment and closed with friction fit injection molded polypropylene closures usually with tamper-evident/resistant features.

Injection stretch blow molded polyester (PET) bottles are often acquired from merchant suppliers for fluid milk with flavored milks on ESL lines, usually in quart or lower sizes.

Gable Top Paperboard Cartons

In half-gallon and below sizes, and especially for the school lunch program (although plastic bottles are penetrating here), the gable top paperboard carton is still popular in the United States for fluid milk products packaging. These cartons are made from liquid resistant virgin solid bleached sulfate paperboard, extrusion coated with low-density polyethylene to impart liquid and water vapor resistance as well as broad range heat sealability. The cartons are delivered to dairies in knocked down sleeve form that permits rapid erection into open top cartons on Elopak, Evergreen, Nimco, or Tetra Rex packaging equipment. On this equipment, the sleeves are snapped open and forced over a mandrel on which a flat bottom heat seal is made by after overlapping the bottom flap of the carton. The erected open top carton is stripped from the mandrel, set upright and filled using a gravity-type filler. The top is heat sealed by folding in a portion of the edges and face-to-face sealing the gable top using pressure and conduction heat. The cartons are sufficiently robust to contain fluid milk for brief distribution periods required, with longer term shelf life impractical because of edge wicking of the paperboard (for longer distribution times, the internal construction is changed). Among the advantages of paperboard cartons are that they may be printed usually with lithographic decoration, or, increasingly with rotogravure or web offset high resolution graphics for consumer display impact. Gable top cartons are not easy to open, but are reasonable to dispense from, and are difficult to reclose properly. They are relatively inexpensive in most small sizes and compete in larger sizes.

SHELF STABLE FLUID DAIRY PRODUCTS

Shelf stability implies heat treatment, either before or after filling to sterilize the contents, that is, render them free of all microorganisms of public health significance and of microorganisms that could cause spoilage under normal conditions of distribution, that is, ambient temperature. (Obviously, altering the water activity of solids could also permit ambient temperature shelf stability.) The term *shelf stable* means

that the contents will not spoil microbiologically, but does not necessarily mean that the product will not deteriorate biochemically and thus retain its initial quality.

POST-FILL RETORTING

Traditional shelf stability is achieved by filling and sealing a barrier package and applying heat sufficient for sterilization, taking account of the rate of thermal death of the microorganisms and the rate of heat penetration. For fluid dairy products which are low acid or pH above 4.6, temperatures required are usually above 250°F which implies retorting, that is, heating under pressure to achieve the requisite temperature, and control of external and internal pressures of the packages during heating and cooling. Canning is the traditional post-fill heat process to achieve ambient temperature microbiological shelf stability.

Canning is largely in cylindrical steel cans which are hermetically sealed mechanically by double seaming a steel end to the body flange after filling. The steel is usually chrome oxide coated and then further coated with an organic material to protect the metal. After closure, the cans are pressure cooked and cooled to create a partial vacuum within the container and reduce, but not necessarily eliminate the rate of biochemical oxidative deterioration. Glass bottles and jars may also be considered as a segment of the canning spectrum. After filling glass containers are hermetically closed with rubber compound lined steel and/or lined or unlined polypropylene closures. The glass packages are carefully aligned and placed in retorts for pressure cooking during which the pressure is carefully controlled with an external overpressure to insure against internal steam pressure blowing off the closures. Further, because of the sensitivity of glass to thermal stresses, careful increase and decrease of temperature is practiced. Relatively little fluid pure dairy product today is packaged in glass in the United States but many coffee beverages some of which contain dairy components are retort packaged in glass bottles.

In Europe, retorting of fluid milk in plastic bottles is not uncommon with HDPE and polypropylene being the package materials of choice. Both are resistant to retort temperatures, but are relatively poor oxygen barriers, and so the contents are subject to significant biochemical deterioration at ambient temperatures. The system is used for relatively short-term ambient temperature distribution.

ASEPTIC PACKAGING

Aseptic packaging is the independent sterilization of product and package and assembly of the components under sterile conditions to achieve ambient temperature shelf stability. Because of the several operations, aseptic packaging is statistically riskier than canning which has a final heat process to compensate for any errors prior to closure. The reason for using aseptic procedures is to significantly reduce the thermal input to the product since it can be heat sterilized in thin film in heat exchangers prior to filling. A second reason is that almost any package material, structure or size may be used. Lightweight flexible or composite materials may be treated by various technologies that render the material sterile without damaging it. Sterilization of the container may be by thermal methods such as dry heat or steam; ionizing radiation; or chemicals such as hydrogen peroxide or peracetic acid. Obviously, when chemicals are used, no residual is permitted.

The most widely used aseptic packaging are paperboard composite bricks or blocks and plastic bottles. Two major and a number of minor paperboard composite systems are commercial using hydrogen peroxide as the sterilant. In the Tetra Pak system, on a presterilized machine, a roll stock web of laminated package material is unwound through a bath of hot hydrogen peroxide and air dried in a sterile environment. On a vertical form/fill/seal machine, the web is formed into a tube in which previously sterilized, cooled dairy fluid is pumped. Induction energy heat seals both a back longitudinal and transverse seam. The latter takes place through the product contents thus eliminating any headspace. The sealed tube is cut from the web and the pouch is formed into a brick shape on a mandrel.

In the SIGCombibloc system, paperboard composite materials are preformed in the converting plant into knocked-down sleeves. At the horizontal form/fill/seal presterilized aseptic packaging machine, the blanks are erected and set upright. Hydrogen peroxide is sprayed into the open top containers and then heated to both raise the operating temperature of the chemical and evaporate the residual. Filling takes place in a horizontal mode with face to face heat sealing of the material using ultrasonic methods. Because the machine is horizontal, multi-lane operation is possible and speed can be as high as 400 packages per minute.

The gable top carton system which continues to be used occasionally in aseptic mode is quite similar

in principle of operation to that of SIGCombibloc except, of course that it is isolated and the materials are sterilized before fillin in the sterile environment.

Aseptic packaging of cups, tubs, or bowls may be accomplished on thermoform/fill/sea or preformed cup deposit/fill/sea systems. In thermoform/fill/sea systems, sterilization may be previous to the dairy in off-line operation or may be on the aseptic packaging machine. On France's ERCA system, the heat of extrusion of plastic sterilizes the webs to be used. On the machine, a protective web of fil is stripped away from the interior surface and the remaining thermoformable web is heated sufficientl to soften it. Sterile air pressure is applied to form the plastic into cup shape in a mold. The open top cup is fille in line and a fl xible closure material is stripped of its protective fil and heat sealed to the flang of the base cups. With a rate of about 20 cycles per minute plus 10 or more cups formed, filled and sealed simultaneously, output can be as high as 300+ per minute for 4–8 ounce capacity cups.

Other thermoform/fill/sea machines such as from Bosch immerse the two barrier plastic webs in hydrogen peroxide and dry them within the machine to achieve sterility. The base plastic web is thermoformed on line prior to filling. Such machines are used for packaging liquid coffee lighteners.

On the Hassia thermoform/fill/sea machine, steam is used both to sterilize the materials and to thermoform the base plastic web into cup shape. Hassia equipment has not been accepted for aseptically packaging low-acid dairy products in the United States (it is accepted for high-acid foods). Thermoform/fill/sea systems generally use coextrusions of polystyrene as the thermoformable structural component and polyvinylidene chloride as the barrier component.

Deposit/fill/sea systems use inputs of preformed cups which may or may not be sterilized on machine. All are closed by heat sealing with fl xible materials which are either predie cut or cut from a fl xible web on the machine. The most widely used are those for liquid coffee lighteners in which the nested cups are presterilized by ionizing radiation and then aseptically transferred to the machine for denesting, fillin with sterile product and heat sealed. Portion Packaging and Purity Packaging systems are similar in operation. Both use thermoformed polystyrene cups and aluminum foil/heat seal coating closures. Generally, the output is maintained under refrigerated conditions despite their sterility, thus accounting for their use of nonbarrier packaging materials.

Hamba machines are popular for aseptic packaging of milk-based puddings using prethermoformed polypropylene cups. Hamba machines have not been accepted for low-acid aseptic operation in the United States, and so products are ESL and must be refrigerated in distribution. The product's quality, however, benefit from the chilled distribution.

Several aseptic bottle filler are commercial with hydrogen peroxide or peracetic acid/hydrogen peroxide as the sterilant of choice. The systems may employ either glass or plastic with polypropylene or HDPE as the preferred materials for short-term ambient temperature distribution, and coextrusions with EVOH for longer-term distribution. Bosch systems have been used for infant formulae; Serac, Stork, Procomac, Krones, Sidel, and Shibuya systems have been used for flui milk products.

For larger size bag-in-box, preformed pouches fabricated from metallized polyester fil and fitte with fillin and dispensing fitment are presterilized using gamma ionizing radiation. On Scholle, LiquiBox, or similar type aseptic fillin equipment, sterile product is introduced through the fitmen which is subsequently sealed. Some web vertical form/fill/sea machines are also operated in aseptic mode.

EXTENDED SHELF LIFE PACKAGING

To prolong the shelf life of flui milk and related dairy products such as fl vored milk, basically two options are currently commercial, aseptic packaging in hermetically sealed barrier containers or ESL packaging followed by chilled distribution. No legal definitio exists specifical for ESL products. ESL products generally fall under existing refrigerated pasteurized (ex PMO) and GMP (CFR) requirements. Each ESL product is the result of matching processing and packaging parameters to meet the acceptable defect level and shelf life. The goal for ESL products is to safely increase shelf life to a goal of up to 90 days of refrigerated shelf life. Acceptable defect levels that do not constitute a public health risk must be established. Defect levels for low-acid ESL products today are generally estimated to be 0.5–2%, largely on paperboard gable top cartons. ESL-packaged products are not *commercially* sterile. Products will *spoil* if not properly distributed, that is, under refrigeration.

The system sequence for ESL encompasses processing, that is, sterilization of product, product cooling, fillin into the package, and closing the package.

For low-acid foods and ESL under refrigeration, GMP regulations are applicable but the product need not be sterile although it usually is sterile. The fill and closing system must be sterilized. The fill and closer must be isolated with interior areas under sterile air usually with high efficiency particulate (HEPA) filters.

The package must be treated to remove microorganisms. Controversy continues on the chemical used, that is, peracetic acid plus hydrogen peroxide or hydrogen peroxide alone. Relevant issues to that must be addressed to achieve the requisite shelf life include treatment chemical concentration and temperature, spray versus wash, upright versus inverted for plastic bottles, drain versus rinse with sterile water and removal of residual.

Alternative sterilization technologies include steam, ionizing radiation used for both bag-in-box and for unit portion coffee lightener cups and their closure materials and ultraviolet radiation.

Packaging is the *only* process where the product needs to be exposed to the "environment." The key to packaging ESL products is to prevent reinfection of the product during fill and distribution. Treatment level must be consistent with required shelf life and depends on the package not being heavily contaminated. Treatment is usually not sufficient to achieve strict microbiological sterility but only sufficient for refrigerated distribution.

Among the ESL packaging systems offered today are Tetra Pak, Evergreen, Elopak for gable top paperboard cartons, Serac, Remy, Procomac, and Kronos for PET bottles; and Scholle and Liquibox for bag-in-box.

With PET bottles, the sterilization protocol depends on the packaging system. In the Sidel/Remy and Procomac systems, bottles may be clean from blowing from preforms followed by aseptic transfer. In the SIPA/Procomac system, bottles may be sterile from fabrication directly from resin plus aseptic transfer and so no further treatment is required.

Closures for plastic bottles are usually polypropylene and must be treated to reduce microbiological load, often with peracetic acid and hydrogen peroxide. Consistent seals are required to maintain product integrity in distribution.

Paperboard cartons for ESL should have barrier in wall and must seal raw edges usually by folding over-skive and hem. The face-to-face fusion heat seal is difficult to open.

PET bottles may have interior the aluminum foil membrane heat sealed to bottle finish die cut from roll or predie cut—removed from stack.

Because the product may not be sterile, microbiological spoilage is possible and biochemical deterioration is probable with oxidation most common.

Reduced temperature during distribution reduces rate and removal of oxygen can reduce oxidative changes.

SOLID DAIRY PRODUCT PACKAGING

Often in dairy product packaging, there is little difference in fill and closing between solid and fluid products. The difference comes later in distribution after the product has set. Thus, from an initial packaging standpoint, packaging is the same, but from a package selection standpoint, it is important to choose structures that will contain the final product and be useful to the consumer. Products such as yogurt and pudding are handled from a packaging operation standpoint as if they were fluids but from a consumer standpoint, their packages must take account of spoonability. In the dairy products field there are numerous soft cheeses, spreads, and so forth, which fall into the same category.

Butter is formed, pressure pumped, and cut into appropriate shapes for pats which may be dispensed onto paperboard trays or overwrapped in aluminum foil laminations in mini-molds or greaseproof paper in full size molds. For delivery into thermoformed cups, however, the butter is directly pumped in fluid form into the cups which are then heat sealed on the flange with aluminum foil. Small quantities are packaged in in-line convolute wound polyethylene-coated paperboard tubs.

Soft cheeses are generally pumped into either thermoformed polystyrene or injection molded polypropylene cups or tubs which are then closed by a combination of aluminum foil heat sealed to the flange friction fit thermoform, with or without tamper resistant ring around the rim. Some soft cheeses are pumped into molds and then cut to be overwrapped, or the cheese may be pumped hot into aluminum foil overwraps with the heat used to reduce the microbiological count. In Europe, considerable quantities of soft cheeses and butter are packaged on thermoform/fill/seal machines using polystyrene as the base cup material and aluminum foil lamination as the heat seal closure.

Some hard cheeses are presented to their packaging machinery as fluid pumped in to tubs, wraps opened within molds, or as thin sheets to be solidified into individual slice wraps. Most cured hard cheeses are sliced and packaged under vacuum or inert gas

flus on one of two basic types of packaging equipment. The most common is Hayssen RT horizontal form/fill/sea using a single web of a lamination containing polyester and nylon with polyethylene as the heat sealant. The cheese in either presliced or block form is delivered to the machine which conveys it to the continuous fl w wrapper forming a long tube around the contents while simultaneously fl wing inert gas to displace the air. A continuous long back seam is formed, and transverse seals are formed between cheese units.

The alternative is a twin web horizontal thermoform/fill/ acuum/ gas flus machine of the Multivac type. The base fil is heated and thermoformed in line creating cavities into which the cheese chunks or slices are placed. Immediately, a top web of fl x-ible material is drawn down over the opening and a partial heat seal is made on the base web flanges. A vacuum is drawn from the interior of the cavity and, if desired, an inert gas back flus introduced. A vacuum causes packaging material collapse on the product while a relatively easy separation of slices if the contents are sliced. A complete heat seal is effected and the individual packages are cut apart. Materials for the base are thermoformable gas barrier plastics such as nylon coated with polyvinylidene chloride (PVDC). The top closure is polyester also PVDC coated with polyethylene heat sealant.

Ice cream may be packaged in bulk for food service scooping and dispensing. Bulk packaging is generally, but increasingly less so, cylindrical spiral wound virgin paperboard coated with polyethylene with heat sealed similar paperboard base and friction fi top, also paperboard. Cylindrical shapes are almost traditional from Sealright, with fillin by flui methods on their equipment. The cylinders may be received in knocked down form to save on packaging material inventory space in which case Sealright equipment is employed to erect the containers. In recent years, the shape has been tending toward rectangular solid, obviously to save space in food service outlets.

Most American ice cream is packaged in coated bleached virgin paperboard half-gallon cartons received in the form of knocked down sleeves. Cartons are automatically erected and fille through one end after which they are mechanically closed by locking the tabs on the cartons. Increasing quantities of consumer size ice cream, particularly the premium types, are packaged in round spiral wound paperboard or plastic containers which are closed by friction fi overcaps, again either paperboard or plastic. Novelties are firs overwrapped on continuous

motion horizontal form/fill/sea equipment with polyethylene-coated paper as the material. Wrapped novelties are then unitized and placed in the ends of opened paperboard folding cartons which are closed by hot melt adhesive.

Numerous other packaging technologies and materials are used commercially and are proposed for dairy product packaging. This dissertation cannot encompass every packaging means available to the dairy packager. A sufficien sampling has been offered to reflec the principal technologies and sufficient basic information has been presented to suggest to dairy packagers alternatives should the suppliers current offerings be less than desired.

FUTURE TRENDS

The most important driving forces facing dairy packagers as of this writing are those from environmentalists. The environmental issue is emotional and replete with misinformation and misperceptions. The issue will continue to mushroom with few predictable paths. Dairy packagers must be cognizant of the volatile situation and be prepared to respond to those having either the force of law or consumer perceptions, however erroneous they might be. Dairy packager suppliers are reactive to environmentalist pressures and will usually be active in assisting their customers. The decision must be made by the dairy on response: do they accommodate every single suggestion from the field regardless of how it disturbs or how much it costs, or do they assume a proactive position and attempt to bring a reasoned approach to the total picture of packaging in the solid waste environment. No matter what stance they take, environmentalism and sustainability will be the top priority for many years.

These imposed activities for dairy packagers should signal that the technologists will be busy with other than technological and marketing advancement in the coming years. Nevertheless, offshore technologies and concepts are not necessarily burdened by American laws and regulations and may be viewed as a resource that might, with caution, be tapped for innovation. There will be continued application of aseptic and ESL techniques to deliver products for refrigerated distribution. The quality of thermally processed dairy products will be better retained by chilled distribution procedures. The quality retention durations for chilled dairy products will be extended by introduction of clean room technologies.

No longer will there be specifi technologies. The new dairy packager will integrate more than one

packaging technology into systems that will deliver a synergy of the benefit from each of the contributing technologies. In the more distant future, the packaging systems will become so sensitive and responsive that they and not the process will be the dominant factors in delivery of quality dairy products. In an era of active packaging, the package will be called upon to sense the contents and to adjust to its technical needs for lower temperature, or aroma enhancement, or microbiological suppression; and to the marketers desire for impact communications with light and sound taking over for mere passive graphics.

Indeed, even as dairy packaging is being questioned, its progress toward a new dimension is already visible on the technological horizon that will be recognized by a perceptive consumer.

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21

Nonthermal Preservation Technologies for Dairy Applications

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INTRODUCTION

Dairy processing is arguably one of the most established and well-developed branches of food-processing technologies. In both scale and sophistication, the productions of commodities such as milk powders, cheese, and pasteurized milk are among the most automated high-throughput food-processing operations, and perhaps among the most intensively studied. Utilization of milk has developed to an efficiency where almost every component has been isolated and, if proven commercially valuable, has had a process for its isolation developed; for example, lactoferrin, a trace but biologically active protein, is now isolated from very large volumes of whey. Indeed, purification technologies based on membrane separation are one of the most active areas of development of what may be called classical dairy processing technologies, particularly in the light of increasing interest in the recovery of

biologically active, or functional components from milk.

Nonetheless, there is currently interest in what may be described as the next generation of dairy technologies. A range of new processing technologies has found niches for other commodity food products on the basis of consumer demands for

- minimal processing, that is, least possible effects of processing on food flavor, appearance, and nutritional quality, yielding fresh-like food products;
- absolute safety of food products, combined with long shelf life and inherent convenience.

In one of the greatest challenges facing food processors today, these two demands of the modern consumer are arguably contradictory; the very means of yielding safe and stable products are those that traditionally have associated changes in food quality. To take an example, preservation by heat has been the staple means of preserving food products for centuries. In the case of milk, heat treatments range from the mild (pasteurization at 72°C and above for 15 seconds) to more severe [ultra-high temperature (UHT) treatment at 130–145°C for 3–4 seconds]. Increasing severity of treatment increases safety and shelf life to the extent that UHT-treated milk may be stored at room temperature for a period of months; however, there is a trade-off in that the sensory quality of milk is inversely proportional to the intensity of the heat applied, to such an extent that consumers in many countries prefer to accept the short refrigerated shelf life of pasteurized milk because of its perceived better flavor.

As a result of such considerations, a number of new technological approaches to the preservation

Table 21.1. New Technologies with Possible Application in the Food Industry**HP processing**

HPH

PEF treatment

Ultrasound

Ultraviolet light

Oscillating magnetic field

High-intensity light pulses

Ohmic heating

Cold plasma

Microwaves

Ozone treatment

HP, high-pressure; HPH, high-pressure homogenisation;
PEF, pulsed electric field

of many food products have emerged; they seek to achieve preservation and inactivation of pathogenic microorganisms without the use of heat treatment, thus yielding minimally processed products that meet the complex demands of consumers; a list of such technologies is presented in Table 21.1. This chapter will first review the principles of application of a number of these so-called nonthermal preservation technologies, particularly with reference to their potential use for milk and dairy products. The focus is on pulsed electric field (PEF), ultrasound, high-pressure homogenization (HPH), and high-pressure (HP) processing. There are few reports of dairy applications of the other technologies to date and therefore they are not discussed here. The challenges in implementing these technologies, in cases where dairy companies identify potential applications that warrant their adoption, are also considered. This is particularly significant in the dairy-processing sector, where scale represents a major obstacle to new technologies, and where apparently promising new approaches based on more conventional technological strategies have not been successfully commercialized in the past. For example, despite the acceptance that the microfiltration of milk can significantly increase its shelf life without loss of sensory quality, the uptake of microfiltration technology for liquid milk has been significantly slower than perhaps would initially have been anticipated.

APPLICATION OF PULSED ELECTRIC FIELDS

Electroporation is a technique that has been used for some years by laboratory microbiologists and

molecular biologists for creating pores in bacterial cell walls, allowing substances such as DNA to be introduced, transferred, or removed. A broadly similar principle underpins the use of PEF in food preservation (Toepf et al., 2007); in PEF, bacterial cells, for example, in a food product, are placed between two electrodes and a PEF is applied. A schematic diagram is shown in Figure 21.1. The field is calculated to be sufficient to kill the cells by opening pores to such an extent that the cell contents are released. PEF treatment typically results in little generation of heat, and is thus a nonthermal process, with generally little effect on food flavor, nutritional quality, or enzymes.

The main commercial successes of PEF have been in fruit-juice-processing applications (e.g., a company in the United States called Genesis Juice has launched a range of PEF-treated juice products). The design of commercial PEF plants favors continuous flow-through processing of liquid products, although applications for solid matrices such as meat have been described. PEF may also have applications in extraction processes, for example, in sugar or fruit extraction.

There have been a number of recently published studies on various aspects of the application of PEF to milk and dairy products (Bendicho et al., 2002; Flourey et al., 2006; Sampedro et al., 2005). Studies of optimal treatment conditions for the inactivation of bacteria including *Listeria innocua* and *Pseudomonas fluorescens* (Fernández-Molina et al., 2006), *Lactococcus lactis* and *Bacillus cereus* (Michalac et al., 2003), and *Staphylococcus aureus* (Sobrino-López et al., 2006) in milk have been described. Microbial proteases in milk may also be inactivated by PEF (Bendicho et al., 2003a,b). It has been shown that raw milk can be stabilized for up to 5 days under refrigeration by the application of optimized PEF conditions (Odrizola-Serrano et al., 2006). Applying PEF to pasteurized milk has been shown to extend the shelf life much more substantially, leading to the possibility of the production of so-called extended shelf-life milk products (Fernández-Molina et al., 2005; Sepulveda et al., 2005). Combining PEF with the addition of antimicrobial agents such as nisin or lysozyme can also result in synergistic inactivation of microbes (Smith et al., 2002).

Changes in casein micelle size profile viscosity (Johnston et al., 2003), and rennet coagulation properties of milk have also been reported (Flourey et al., 2006). Cheddar cheese has been made from PEF-treated milk (Sepulveda-Ahumada et al., 2000).

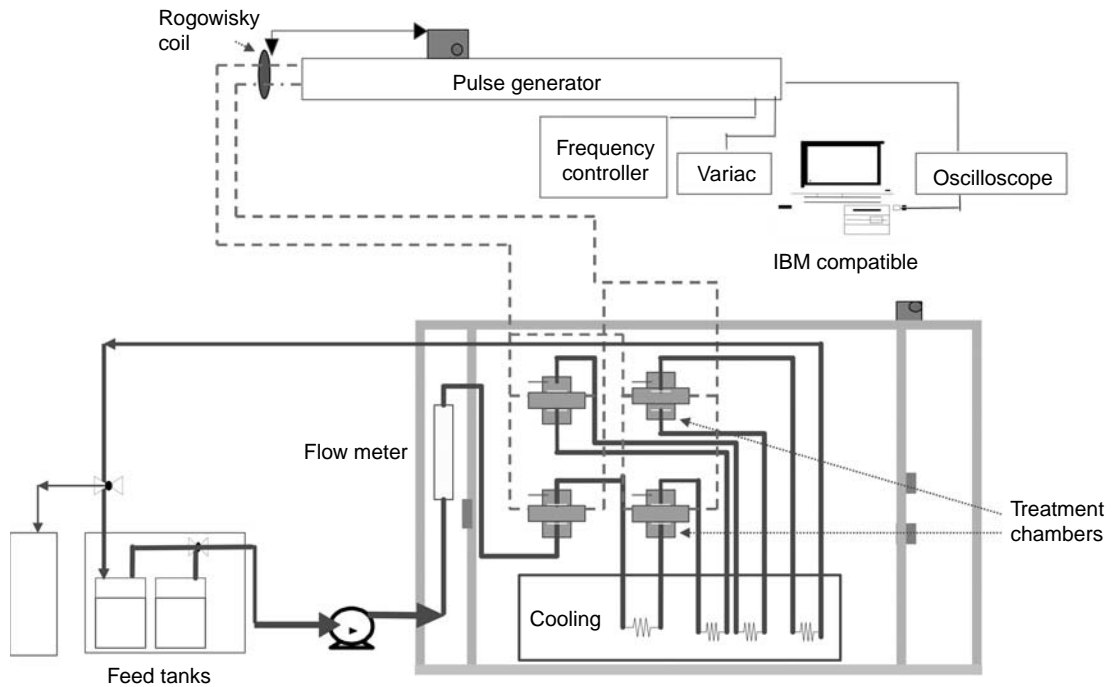


Figure 21.1. A schematic diagram of a pulsed electric field (PEF) system. The food product to be processed is pumped through treatment chambers to which electric field pulses are applied by means of a pulse generator.

Potential safety concerns for the operation of PEF plants include the generation of gas bubbles (which can be controlled by operation under elevated pressures) and electrical arcing (which may occur at very high voltages).

ULTRASOUND TREATMENT

Another emerging technology that has been applied to dairy products with some success to date is high-intensity ultrasound; such treatments involve generating waves of high power levels ($10\text{--}1,000\text{ W/cm}^2$) and low frequencies ($\leq 0.1\text{ MHz}$), and passing such waves from the generating probe (or sonotrode) through gas, liquid, or solid materials. When such waves pass through materials, cavitation occurs, leading to generation of bubbles and rapid collapse, generating localized shock waves and fluctuation in temperature and pressure as well as the production of free radicals. Whereas applications involving the use of ultrasound for cleaning have been described in the dairy industry, ultrasound may also be of interest as a processing tool for the inactivation of bacteria (for

reviews, see Knorr et al., 1993, 2004; McClements, 1995; Villamiel et al., 1999). Inactivation of bacteria, including *Listeria monocytogenes* (D'Amico et al., 2006), has been demonstrated following ultrasound treatment of milk.

Enzymes may also be inactivated by ultrasound, including alkaline phosphatase in milk (Ertugay et al., 2003; Villamiel and de Jong, 2000a), although there have also been reports of increased enzyme activity following ultrasound treatment (McClements, 1995). Ultrasound may also be used effectively as a homogenization technology, yielding very fine emulsions but at very high-energy consumption (Abismail et al., 1999; Behrend et al., 2000; Jafari et al., 2007; Wu et al., 2000). Systems for the continuous processing of liquid milk using ultrasound have been described, and may allow a combination of microbial inactivation with homogenization (Villamiel and de Jong, 2000a,b).

Hurdle treatments combining ultrasound, elevated temperature, and pressure (manothermosonication) have been described and have been found to exploit synergistic inactivation mechanisms for bacteria;

such treatments have also been applied to milk for yogurt making, and have been found to yield stronger gel structures (Vercet et al., 2002). The production of hydroxyl radicals by ultrasonic treatment may induce cross-linking of food components (Wan et al., 2005).

HIGH PRESSURE HOMOGENIZATION

Whereas homogenization of milk to delay or prevent cream separation has been practiced for over a century, in recent years, there has been significant interests in applying homogenization at very high pressures (100–300 MPa, compared with 10–30 MPa for conventional homogenization), either in equipment similar in design to conventional homogenizers, or using specialized equipment such as microfluidizers where homogenization is achieved by impinging HP liquid jets (Jafari et al., 2007). HPH can obviously yield very fine emulsion particle sizes with narrow particle size distributions, but potential commercial interest is likely to be driven by the effects of such high dynamic pressures, and associated physical effects such as shear, cavitation, and rapid temperature changes, on microorganisms, proteins, and enzymes in milk. Several recent studies have characterized these changes for HPH technology in some detail, demonstrating microbial inactivation with some minor changes to whey proteins and enzymes in milk (Datta and Deeth, 1999, 2003; Hayes and Kelly, 2003a,b; Hayes et al., 2005; Picart et al., 2006; Thiebaud et al., 2003), and products such as yogurt (Lancioti et al., 2004; Serra et al., 2007) and ice cream (Hayes et al., 2003) have been made from HPH-treated milk and their properties characterized. There have also been a number of studies of emulsification using microfluidization (Dagleish et al., 1996; McCrae, 1994; Morgan et al., 2000; Robin et al., 1992, 1993).

HIGH PRESSURE PROCESSING

Although it is usually regarded as a novel processing technology, HP processing has actually been around for over 100 years, and was indeed first applied to milk not long after Pasteur's seminal studies on the preservation of food products, including milk, by the application of heat. Around the turn of the twentieth century, Bert Hite a chemist based in the Agricultural Experiment Station laboratories at West Virginia University built a steel cylinder in which he subjected milk to extremely high pressures (over

1,000 times atmospheric pressure) and showed that shelf life could be extended. Hite also demonstrated that several food-borne pathogenic bacteria could be inactivated by pressure, but safety fears arising from the propensity of his equipment to explode while at pressure, spraying pathogens and metal, led to the end of this promising investigation, which was genuinely ahead of its time.

It was almost 90 years later that HP-treated food products became commercially available, with the launch of jams, fruit products, and other commodities in Japan, facilitated by key developments in equipment operation and reliability in HP units developed for nonfood applications such as metals and ceramics.

Modern HP processing equipment subjects samples to pressures in the range 100–1,000 MPa (or 1,000–10,000 times atmospheric pressure). The samples are usually processed in-package in a batch-wise processing system, being immersed in a larger volume of pressure-transmitting medium (e.g., oil, water, or emulsion, depending on the system being used), which is then subjected to external pressures, typically using HP pumps or pistons; the surrounding medium then transmits the pressure to the sample, assuming that the packaging is flexible enough to withstand the external pressure applied. A schematic diagram is shown in Figure 21.2. The vessel for treatment is usually cylindrical and made either of extremely thick monolithic steel or thinner-walled steel with wire-winding to add structural strength. Compared with heat, HP acts instantaneously and uniformly throughout a mass of food independent of size, geometry, food composition, mass, and time, which insures the absence of dead spots and localized over-processing, which are problematic with other treatments. Commercial treatments are generally applied at room temperature because of the expense and energy involved in adjusting the temperature of such large quantities of material; however, treatments at high (e.g., 90°C or above) or low (e.g., <5°C) temperatures may be of interest for spore inactivation or modification of freezing/thawing/ice crystal structure, respectively.

For the last 15 years or so, there has thus been a resurgence of interest in HP technology, for both commercial and scientific reasons (Hogan et al., 2005). In research terms, more attention has arguably been paid to milk than to any other food product; a search of the ISI Web of Knowledge in June 2007 using the terms “milk” and “high pressure” returned 979 articles, compared with 370, 475, 45, and

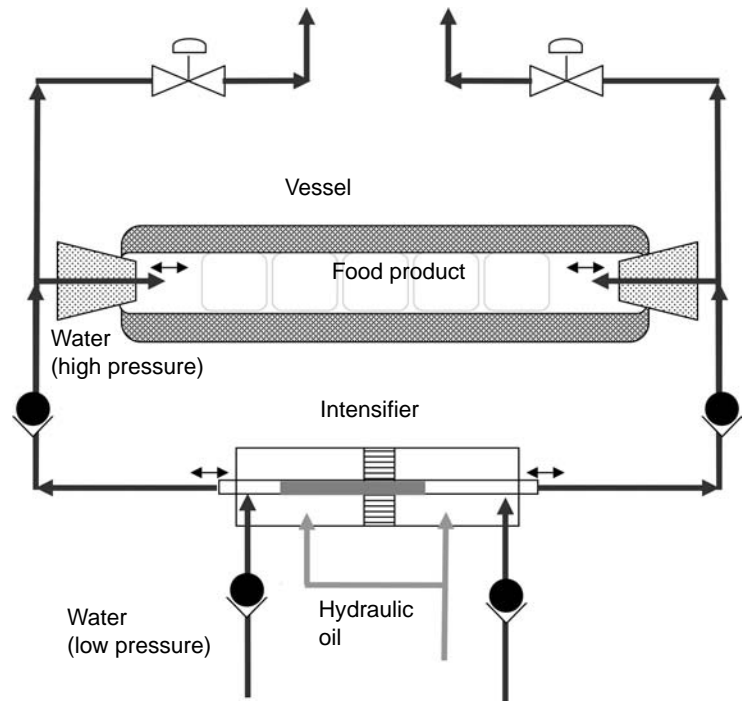


Figure 21.2. A schematic diagram of an high-pressure (HP) system. An intensifier driven by hydraulic oil raises the pressure of water in an HP vessel containing the food product to be processed.

509 records for “high pressure” and “juice,” “meat,” “oyster,” and “fruit,” respectively. However, it is interesting to note that the first HP-treated commercial dairy products came to market significantly later than the other product categories mentioned. This may be attributed partly to challenges of commercialization, which are discussed later in this chapter, and partly to the complexity of the effects of pressure on the milk system, which are now considered.

For many food products, HP processing gives the optimal effects of conventional processing approaches such as heating (e.g., inactivation of pathogenic and spoilage microorganisms, inactivation of undesirable enzymatic activity) without the drawbacks associated with conventional technologies (e.g., loss of flavor or nutritional quality, or changes in appearance); the archetypal product in this regard is fruit juice. For certain niche applications (e.g., guacamole, oysters), it is clear that HP represents the first realistic processing solution for shelf-life extension without major changes to product characteristics and consumer acceptability. Therefore, for foods where thermal pasteurization is not an option (due to flavor, texture, or color changes and degradation in the nutritional quality of heat-sensitive

products), HP may be beneficial and can extend the shelf life by up to two- to threefold as compared to a nonpasteurized counterpart and can improve food safety. In addition, HP may have desirable effects on food characteristics such as texture (in the case of meat) or other effects that are difficult to achieve otherwise (e.g., shucking of oysters).

However, for none of the cases described above does HP result in changes that are not seen as a consequence of traditional technologies, but merely achieves them more easily or without concomitant undesirable effects, and hence the route to commercialization can be readily predicted. In the case of milk, this is simply not the case; effects seen following HP treatment of milk and dairy products are frequently unpredictable and unique (for reviews, see Huppertz et al., 2002; López-Fandiño, 2006a; Trujillo et al., 2002).

MACROSCOPIC CHARACTERISTICS OF MILK

Perhaps one of the most striking effects of HP in the dairy context occurs when skim milk is treated at pressures above around 300 MPa; following

release of pressure, such milk appears to be considerably more translucent, more closely resembling whey. Such effects are not seen when whole milk is treated because of the light-scattering of the fat globules; however, whole milk treated at low pressures (≤ 250 MPa) exhibits a more rapid separation of a much thicker cream layer than that formed in un-homogenized raw milk, whereas treatment at higher pressures (≥ 400 MPa) results in milk that creams only very slowly, despite no changes in fat globule size (Huppertz et al., 2003).

When pressure-treated milk is used for cheese-making, changes in rennet coagulation time, gel strength, curd yield, and cheese ripening have been reported; depending on the conditions used, these changes may be positive or undesirable for cheesemakers (Huppertz et al., 2004a). Similarly, the acid gelation properties of HP-treated milk are different from those of normal milk, with firmer gels that are less prone to syneresis (Harte et al., 2003; Needs et al., 2000; Penna et al., 2006; Walsh-O'Grady et al., 2001).

Cheese itself may also be treated under pressure (O'Reilly et al., 2001; Stewart et al., 2006) and functionality of cheese can be improved (O'Reilly et al., 2002). Indeed, one of the first patents concerning the application of pressure to dairy products was from Japan and it suggested that HP treatment of fresh Cheddar cheese for 3 days at 50 MPa would result in a flavor and an amino acid content close to those of mature cheese, offering the possibility of dramatic acceleration of cheese ripening; however, detailed scientific studies failed to confirm this as a commercially relevant possibility (O'Reilly et al., 2000a,b, 2002), although several studies have confirmed more modest degrees of acceleration of ripening using higher pressures and shorter times (O'Reilly et al., 2003).

There has more recently been interest in using HP to arrest cheese ripening, by inactivation of the microorganisms and enzymes that catalyze biochemical changes in the cheese, offering the potential to extend the shelf life of certain cheese types at their optimal quality. This is an area of considerable potential interest, and a company in Spain has recently commercialized a cheese product that is rendered microbiologically stable by the application of pressure.

There are a few reports on the implications of HP treatment for products other than milk and cheese; however, this may be due to relative youthfulness of

the field rather than lack of potentially interesting outcomes.

EFFECTS OF PRESSURE ON MILK CONSTITUENTS: THE UNDERPINNING MECHANISMS

The changes in milk and cheese properties summarized above are due at least in part to the complex effects of pressure on milk proteins (Considine et al., 2007; Huppertz et al., 2006a; Patel et al., 2006). Many changes in the properties of milk treated at temperatures above those used for pasteurization result from the thermal denaturation of β -lactoglobulin, which exposes a highly reactive thiol group that can lead to complex formation with a range of milk proteins containing disulfide bonds, including β -lactoglobulin itself, α -lactalbumin, and the casein micelle, due to interactions with κ -casein at its surface. HP treatment also results in whey protein denaturation, with β -lactoglobulin being denatured at pressures > 100 MPa, while the other whey proteins are more pressure-resistant (Huppertz et al., 2004b, 2006a,b).

However, although the caseins are generally resistant to changes at the temperatures used in dairy processing, except for interactions with denatured whey proteins and perhaps coagulation and some hydrolysis under retorting conditions, very significant changes occur under pressure. At pressures > 200 MPa, studies of light-scattering using pressure vessels with sapphire glass windows have shown that the casein micelles dissociate under pressure because of solubilization of micellar calcium phosphate and disruption of electrostatic interactions (Huppertz et al., 2006b).

However, following pressure release from around 250 MPa, to an extent depending on the duration of treatment, the micelles reassociate but yield particles that are up to 20% larger than the micelles in untreated milk at room temperature (and more so at 40°C); hydrophobic bonding seems to be important in the formation of these aggregates (Huppertz et al., 2004b, 2006b). Following treatment at pressures > 300 MPa, the particles that reform on the release of pressure are significantly smaller than native micelles, probably because of more extensive solubilization of the calcium phosphate, which is a key agent of micellar integrity (Huppertz et al., 2004b, 2006b). The latter changes are responsible, because of the concomitant reduction in light scattering, for the changes in the appearance of skim milk. The alterations in the

creaming properties of whole milk are apparently due to changes in the agglutination mechanisms in milk, perhaps because of pressure-induced changes in the structure and functionality of immunoglobulin molecules (Huppertz et al., 2003).

There are only a few studies on the effects of pressure on lactose and milk fat; however, evidence to date seems to suggest little effect on these constituents.

TEXTURE MODIFICATION USING HP

Although the main focus of HP was initially food preservation, it has recently been of interest for its potential to improve food texture and to create new products (Datta and Deeth, 1999, 2003; Huppertz et al., 2002). In contrast to heat treatments, HP is known to affect noncovalent bonds, such as hydrophobic and hydrogen bonds, but to have little effect on covalent bonds. As a result, the large biomolecules, such as proteins and polysaccharides, in which noncovalent bonds play a major role in maintaining structure and function, are affected most by HP (Masson, 1992; Messens et al., 1997), whereas smaller molecules such as vitamins and flavor are minimally affected because covalent bonding is dominant in maintaining their structural integrity. HP may be used to change the molecular structure of proteins to give novel properties that are not possible via traditional methods. In this way, HP provides an opportunity to create and control food texture in protein- or starch-based foods. In some cases, pressure can be used to form protein gels with unique texture properties and increased viscosity without using heat (Dumay et al., 1994; Famelet et al., 1998; Keim and Hinrichs, 2004; Patel et al., 2005; Van Camp et al., 1995a,b).

MICROBIOLOGICAL CONSEQUENCES OF HP TREATMENT

As stated earlier, one of the advantages of HP treatment as a food-processing technology is its ability to inactivate spoilage and pathogenic microorganisms. In the case of milk and dairy products, numerous studies have elucidated conditions under which various microbes are killed by HP treatment (Huppertz et al., 2006c). However, it is unlikely that microbiological control alone would be sufficient to warrant HP treatment of liquid market milk, as its advantages relative to cost will not compete with established technologies such as pasteurization and UHT treatment. Also, a key remaining obstacle is that HP treatment

is relatively ineffective against spore-forming bacteria, at least at moderate temperatures or in the absence of other hurdles (e.g., nisin). Recent studies have helped elucidate the mechanism by which milk seems to offer particular protection to the inactivation of microorganisms suspended therein, for example, relative to simple buffer systems (Black et al., 2007).

POTENTIAL DAIRY APPLICATIONS OF HP

As mentioned earlier, for dairy systems, HP is more extensively studied, and offers more potential product opportunities at this point, than the other nonthermal preservation technologies discussed above (Rastogi et al., 2007). A number of these dairy applications are identified in the published literature and are listed in Table 21.2. Despite the abundance of opportunities, the commercial uptake of nonthermal preservation technologies for dairy processing is still relatively low (Devlieghere et al., 2004; Rastogi et al., 2007; Torres and Velazquez, 2005). Some of the key challenges to their adoption by the dairy industry are considered in the remainder of this chapter.

COMMERCIALIZATION AND IMPLEMENTATION CHALLENGES

Cost is usually the most decisive consideration in the implementation of nonthermal preservation technologies, such as HP processing. Capital expenditure is invariably required and the operating costs are typically higher than for traditional preservation technologies. This means that the nonthermal technology must offer a distinct benefit to the producer or consumer, which must be justifiable in terms of the additional value created. A traditional technology that gives a similar outcome is likely to be a lower-risk lower-cost alternative.

As the capital and operating costs of the nonthermal technologies described above are well characterized, the minimum breakeven cost that has to be recovered before the novel product becomes profitable may be calculated. An indicative example for HP processing is shown in Figure 21.3, for a variety of typical processing conditions (operating pressure and holding time at target pressure) and with a 2-year payback on capital expenditure (a typical food industry requirement). Financial viability requires the value of the enabled opportunity to be positioned above the breakeven lines and these in turn are strongly

Table 21.2. Summary of Applications of HP Processes to Dairy Products

Product Application	Potential New Technological Approach	Reference
Extended shelf-life milk	HP-treated milk to inactivate spoilage microorganisms and with improved sensory, vitamin retention, and so on	Hite (1899); Mussa and Ramasamy (1997); Sierra et al. (2000)
Reduced fat cheese	Improved body, softer texture, and better taste compared with that made by conventional methods because of high moisture retention	Molina et al. (2000)
Cream	Improved whipping functionality	Eberhard et al. (1999)
Ice cream with improved texture	Reduced fat globule crystal sizes in ice cream mix	Keenan et al. (2000)
Functional ingredients from milk proteins	Altered functional properties by conformational changes to the protein structure including foaming, emulsifying, gelling, and water-binding capacities of the proteins, retaining functionality of iron-lactoferrin	Johnston et al. (1992, 1993); Liu et al. (2005); López-Fandiño (2006a,b); Palmano et al. (2006)
Humanized milk, baby food, or hypoallergenic dairy products	Selective denaturation and removal of β -lactoglobulin	Balci and Wilbey (1999); López-Fandiño (2006b)
Acidified dairy beverages with bioactive ingredients	Inactivation of spoilage microorganisms while preserving bioactivity, retaining bioactivity of iron-lactoferrin	Carroll et al. (2006); Palmano et al. (2006)
Improved cheese manufacturing efficiency	HP-treated milk for reduced coagulation time and increased curd-firming rate	López-Fandiño et al. (1996); O'Reilly et al. (2001); Trujillo et al. (2002)
Increased cheese yield	HP-treated milk to incorporate more whey proteins and moisture	López-Fandiño et al. (1996); Drake et al. (1997)
Extended shelf life of fresh cheese	Inactivation of spoilage microorganisms while maintaining textural properties	Capellas et al. (1996); Trujillo et al. (2000)
Reduced production costs of shredded cheese	Instant change in cheese microstructure to that of ripened cheese after HP	Serrano et al. (2005)
Yogurt with extended shelf life	Selective inactivation of spoilage organisms while maintaining starter counts	Carroll et al. (2004)
Yogurt with improved texture	HP-treated milk for β -lactoglobulin interaction with α_{s2} -casein as well as κ -casein	Johnston et al. (1993); Datta and Deeth (2003); Lanciotti et al. (2004)

HP, high-pressure.

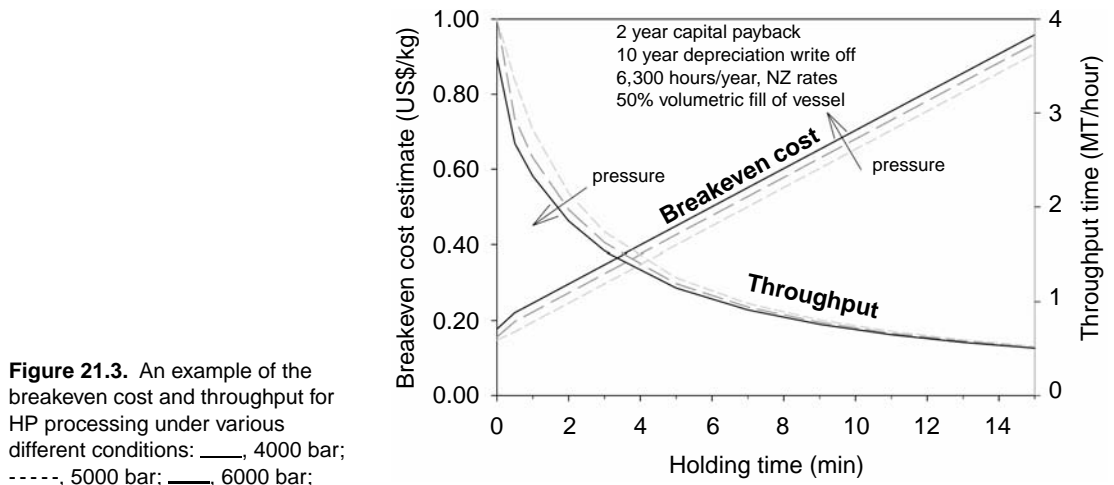


Figure 21.3. An example of the breakeven cost and throughput for HP processing under various different conditions: —, 4000 bar; ----, 5000 bar; —, 6000 bar;

dependent on the processing conditions required to deliver the desired outcome. The processing conditions are typically available from the research literature or can be established in preliminary trials. In the example of Figure 21.3, the minimum breakeven cost is about US\$0.15 per kg and this increases about threefold when the holding time increased from 3 to 15 minutes. In some situations, the added cost is recovered through greater savings in other production or distribution costs (for example, Serrano et al., 2005). Alternatively, the additional cost is recovered through a higher selling price for an added-value product, in which case it should be further scaled by the appropriate ratio of retail price to production cost, to estimate a price that could be assessed for potential market response. Business models such as contract processing have been applied to HP technology to remove the capital barrier to the adoption of the technology (e.g., <http://www.pressurefresh.com>). The compromise is an increase in the breakeven cost because of the added processing cost.

Nonthermal preservation technologies are also well served by several contract research and development facilities, where problem-owners and solution-providers can collaborate with minimal risk. Examples include the Innovative Foods Centre, Food Science Australia and the National Center for Food Safety and Technology (NCFST), USA. The links between industry and academia are also particularly strong [for example, through the Non-thermal Processing Division of the Institute of Food

Technologists (IFT), USA; the Center for Advanced Processing and Packaging Systems (CAPPS), USA; and the European Federation of Food Science and Technology (EFFoST)].

The benefit of nonthermal preservation technologies such as HP in dairy applications include reduced microbial spoilage and improved functional properties, as discussed earlier. The benefit of reduced spoilage can include increased market reach (including export opportunities) and improved utilization of existing capital assets. Examples of competing technologies for processors might include membrane filtration for fluid products and preservatives for gel products. Benefit of improved functionality can include creation of novel textures, where alternatives might include texture modifier such as starches and gums, or preservation of bioactivity, where alternatives might include dehydration and membrane filtration. The justification for a nonthermal technology to give improved food quality at higher cost (particularly in circumstances where there are proven lower-cost/lower-quality alternatives) is more challenging than in a situation where a preexisting market or manufacturing need is uniquely addressed. A final question is whether the technology applies satisfactorily across the complete range of product formats as required, for example, the range of serving sizes and flavors; a solution that is too narrow in scope may have limited appeal.

Nonthermal preservation technologies present some issues when integrating into an existing operation, which is invariably more of a challenge than

beginning from a purpose-built factory. The first step is to establish the process fit with existing operations, such as filling and packing, to avoid misalignment in capacity and throughput. Continuous processes such as ultrasound or PEF are more straightforward to integrate than inherently batch processes such as HP processing. Impediments may arise when the throughput of the novel technology is rate-limiting. As HP processing is typically carried out after packing and other technologies such as power ultrasound and PEF are carried out before filling the ability to integrate with filling and packing operations is essential in all cases. The maximum throughput of an HP machine as given in Figure 21.3 is approximately 1,000–4,000 L/h, which is certainly within the scope of a modest packing line. An example of an integration challenge is increasing cheese yield by HP processing milk at 400 MPa for up to 20 minutes (Huppertz et al., 2004a; López-Fandiño et al., 1996). The maximum capacity of this process (estimated from Fig. 21.3) is about 500 L/h, creating potential delays in filling the cheese vat, with drawbacks that may outweigh the benefit of HP. Another example is the use of either power ultrasound or HP processing in addition to the standard milk heat treatment for yogurt manufacture. These processes produce a yogurt with improved functionality (including reduced syneresis and increased yield stress). A pressure process of 400–500 MPa for 5 minutes for this application (Harte et al., 2003) has a maximum capacity (estimated from Fig. 21.3) of about 1,000 L/h, which in turn limits the fermentation batch size or increases the production time. In comparison, the power ultrasound process of 450 W/150 g of a product for 6 minutes at 20 kHz for this application (Wu et al., 2000) corresponds to a power density for scale-up to a continuous process of about 3 kW/L, which exceeds the output of the typical commercial sonotrode. Alternative scale-up strategies such as increasing integrated energy input come at a substantial loss of capacity (from increased residence time) and additional sonotrodes add capital cost and complexity.

PRODUCT DEVELOPMENT CHALLENGES

The full impact of the nonthermal technology on all aspects of quality over the shelf life should be understood, including the impact on sensory, physical, enzymatic, and microbial characteristics, comparisons with traditional processes (a part of the process toward regulatory acceptance in many markets), and

stability of nutritional components. Such investigations should be at a significantly high level so that the findings can be applied confidently at the industrial scale and failure rates can be established. The nature of dairy processing is to manufacture a consistent product from a variable raw material and the robustness of any potential nonthermal technology should be considered in this context, for example, by evaluating product manufactured from different lots or at different times in the season. The implications of failure to deliver the specified outcome on product quality must also be understood. A product that is too sensitive to processing parameters or processing exceptions may also be difficult to manufacture at scale.

Shelf-life evaluations should be on the actual foods, formulations, and ingredients to capture the often confounding effects of specific ingredients [e.g., the presence of sucrose in a formulation can affect microbial inactivation (Chauvin et al., 2006; Gao et al., 2006) and protein denaturation (Dumay et al., 1994) in HP-treated products]. Food matrices have been reported as offering protection from microbial inactivation compared with buffer solutions (Balasubramaniam et al., 2004), especially in the case of milk (Black et al., 2007). Challenge trials are an important tool at all stages in product development. The final food product is a critical consideration in the selection of microorganisms, either food isolates or pressure-tolerant surrogates for the microorganism of concern (Balasubramaniam et al., 2004). There is also evidence that nonthermally processed microorganisms can recover if sublethally injured (García-Risco et al., 1998). Furthermore, tailing phenomena, heterogeneity within a population, and failure to follow first-order inactivation kinetics may occur (Chen and Hoover, 2003a,b). Sublethal injury and subpopulation tailing are more likely to appear (if they are an issue) at scale-up and in shelf-life studies on the actual food product than in laboratory experiments. Survival and recovery of sublethally injured cells may be a particular concern where product is recycled for repeat processing, as future tolerance may develop (Hauben et al., 1997).

The relationship between processing performance and equipment design must be properly understood for successful scale-up and interpretation of research data at laboratory scale (Balasubramaniam et al., 2004). Examples of scale-related design issues include flow cell design in PEF, pump-up rates and pressurizing fluid selection in HP processing (Balasubramaniam et al., 2004; Ting et al., 2002), and

power intensity and density in power ultrasound. The reliability of the processing technology is also important in terms of the ease of operation and maintenance under industrial conditions, which are much more demanding than laboratory conditions. Of course, reliability should improve as technology matures.

Often, research experiments and variables are not selected from the perspective of commercial application, and reporting of experimental design, equipment performance, and methodology can create difficulties in applying findings from one situation to another. Guidelines for conducting microbial research on HP processes have been published (Balasubramaniam et al., 2004), in an effort to promote consistency and interpretability.

Packaging is a consideration in preserving product quality over an extended shelf life, and some technologies, in particular HP, may place constraints on packaging material properties. Special packaging solutions may add cost and may create operational difficulties if additional stock-keeping units require alternative packaging specifications.

Microbial inactivation and growth concerns also constrain opportunities for dairy applications. Hurdle technologies and combinations of technologies offer promise in overcoming some of the limitations of individual technologies (Barbosa-Cánovas et al., 1998; Crawford et al., 1996; Leistner, 1992, 2000; Raso et al., 1998; Ross et al., 2003; Yousef, 2001; Williams, 1994), although these may add complexity and cost. Microbial hurdles such as pH and storage temperature are particularly critical in general; an example of this for HP processing is shown in Figure 21.4. Although much of the scientific research on HP processing of dairy systems, both microbiological and physicochemical, is in the near-neutral pH range, shelf-stable neutral dairy products (such as a shelf-stable long-life alternative to UHT milk) are not yet possible by HP processing alone because of the inability to inactivate bacterial spores. Chilled low-acid products such as long-life fresh cheeses may be achievable depending on the behavior of the microorganism of concern under processing and storage. In contrast, acidified dairy products with chilled distribution (such as long-life yogurt with live cultures) are feasible and acidic products with ambient distribution (such as fruit milks and smoothies) may also be possible if microbiological stability can be achieved, as dairy spores typically do not germinate and grow under such conditions. Combined thermal-pressure processes that offer the promise of spore inactivation are being developed (see reviews by Matser et al., 2004;

Ross et al., 2003). This approach is based on combining pressure and the modest heating effect caused by compression (i.e., adiabatic heating) to achieve bacterial spore inactivation at lower heat inputs than comparative thermal processes such as retorting; the detrimental effects of heating on the food are mitigated in this way. Some *Bacillus* spore inactivation may occur at above 500 MPa at 60°C (Margosch et al., 2004; Scurrah et al., 2006) but the extent of inactivation depends on species and strain. In general, the microbial lethality of nonthermal processing technologies is greater under acidic conditions, as it is for thermal processes (Balasubramaniam et al., 2001; Barbosa-Cánovas et al., 1999; Hoover et al., 1989). As mentioned earlier, elevated temperatures also improve the microbial lethality of HP (Earnshaw et al., 1995; Patterson et al., 1995; Rademacher et al., 1998).

The regulatory environment for HP dairy products reflects the importance of pH and storage temperature, as shown in Figure 21.4. For example, in the United States, process validation would be required for products regulated by hazard analysis and critical control points (HACCP), but there are limited regulatory issues with refrigerated or acidified shelf-stable products. In contrast, low-acid shelf-stable products would require a process to be filed. In the European Union (EU), HP comes under the Novel Foods Regulation (EC) 258/97, and the first premarket authorization for an HP-pasteurized product (acidic and chilled) was given in 2001, with a second following on substantial equivalence. There are also a number of prepared meat products (chilled but low-acid foods) on the market in both the United States and the EU, with the U.S. Department of Agriculture not objecting in the former and a market history established in the latter. The main consideration, both in the EU and in other countries such as Australia and New Zealand (for pasteurized dairy products), is whether the novel process changes the product, with the processor having the responsibility to establish this.

Opportunities that require the replacing of thermal pasteurization of raw milk are much more complex to develop, as no new technology described can fully meet all of the outcomes of thermal pasteurization. However, there are promising niche opportunities in fresh cheeses or raw milk cheese where the proposition is improved sensory characteristics (Buffa et al., 2001; Capellas et al., 2001; Daryaei et al., 2006; Molina et al., 2000; Sandra et al., 2004).

There can be substantial differences in the effects of nonthermal technologies on bacterial and fungal

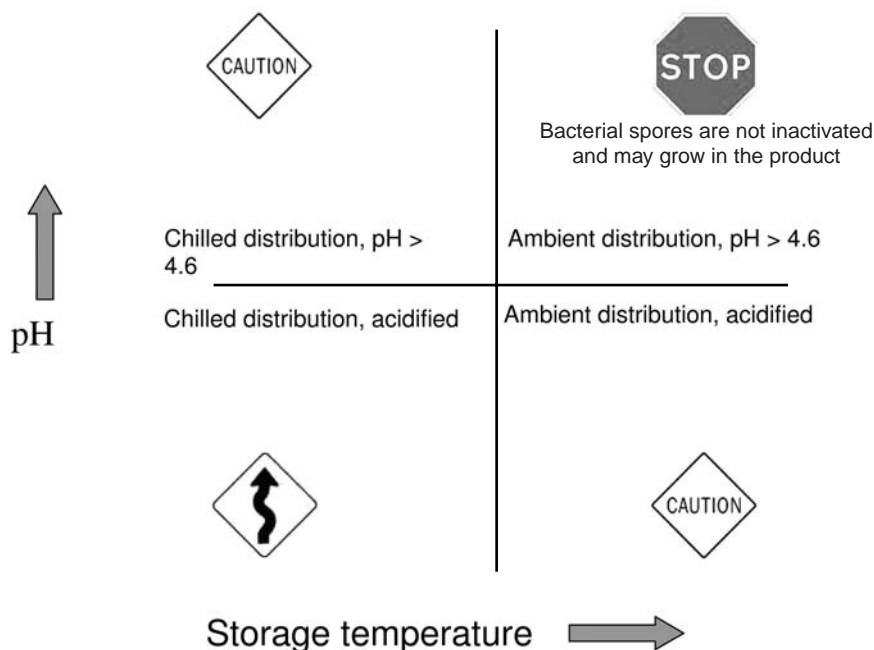


Figure 21.4. Novel preservation technologies that are ineffective against bacterial spores have limited value for ambient distribution of near-neutral pH dairy products. Chilled distribution of acidified dairy products is relatively low risk (e.g., long-life yogurt). In the other two segments, microbial control is a key component of product development.

spores (Butz et al., 1996; Gould and Sale, 1970; Leistner and Gould 2002) and indigenous milk enzymes (Rademacher and Hinrichs, 2006; Scollard et al., 2000a,b), compared with heat treatments (e.g., UHT). Native milk enzymes may cause quality degradation during storage if not sufficiently inactivated by the nonthermal process. These enzymes include plasmin, protease, lipase, esterase, xanthine oxidase, and phosphatase (Claeys et al., 2003; Scollard et al., 2000a,b). This may limit applications of nonthermal technologies to dairy products, even in foods in which enzyme activity is regarded as being beneficial to quality (e.g., cheese-making), as flavor development can be distorted. Enzyme–substrate interactions and reaction kinetics may also be altered, particularly by HP (Rademacher et al., 1999), and this may offer the possibility of novel reaction products.

SUMMARY

Nonthermal preservation technologies may be used to create a wide range of novel and desirable effects in dairy systems that may lead to innovative products,

processing improvements, and new business opportunities. Although the wealth of research knowledge points to a bright future for select technologies, and HP in particular, there are a number of challenges in realizing the commercial potential. The best potential dairy candidates for commercialization are likely to have the following features:

- a unique value proposition that cannot be delivered by traditional processing or through reformulation (e.g., bioactivity, nutritional, or sensory);
- a solution to a preexisting problem or unmet market need;
- a reliable manufacturing process operated under conditions that are cost-competitive, with sufficient throughput or capacity and feasible integration with the other steps in the manufacturing process; and
- acceptable microbiological quality at scale, preferably a pH below 4.6, and/or chilled distribution and other effective formulation hurdles to microbial growth as appropriate (e.g., salt, sugar); examples might include acidic and

fruit milks, yogurt, and yogurt drinks and smoothies.

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22

Management Systems for Safety and Quality

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Essential Elements of Food Ethics
Corporate Business Culture, Market Economy, and
Quality Movement
Current Good Manufacturing Practices
Principles and Essential Elements
Dairy Food Safety Systems
 Implementing HACCP (Where Rubber Meets the Road!)
 Get Organized
 HACCP Team (Where Is My Dream Team?)
 Describe Your Food, Its Distribution Dynamic,
 and Intended Use by the Consumers
 Develop a Flow Diagram and Physically Verify That the
 Flow Diagram Is Current and Accurate
Hazard Analysis
 Principle 2: Determine Critical Control Points
 Principle 3: Establish Critical Limits
 Principle 4: Establish Monitoring Procedures
 Principle 5: Establish Corrective Actions
 Principle 6: Establish Verification Procedures
 Principle 7: Establish Record Keeping and
 Documentation Procedures
Deming's Quality Doctrine
Kaizen, Six Sigma, and its Relevance to Food
 Safety and Quality
Codex Standards and the Global Food Trade
Main Functions of Codex Commission
Functional Structure of Codex
General Committees
Commodity Committees
Conclusions
References
Bibliography

ESSENTIAL ELEMENTS OF FOOD ETHICS

Food industry has continued to evolve over time.
The U.S. food industry is a multi-billion dollar en-

terprise showing continued growth and expansion. Modern food industry has embraced fundamental ideals of providing pure, safe, nutritious, and wholesome foods. Through years of experience, knowledge exchange and healthy dialogue among various stakeholders (comprising food industry, regulatory agencies, academia, judiciary, consumers, and society at large), now it has become well accepted that consumers have unalienable right to receive safe foods. When consumers buy products from the market shelf, his confidence in safety and wholesomeness of food item he buys must be upheld at all times. Toward this end, primary responsibility for providing safe and wholesome food rests with the manufacturer. The realization that consumption of food has a bearing on health and well-being, delivering safe and nutritious products to consumers on a consistent basis becomes professional and ethical responsibility of food manufacturers. Since multitude of factors influence quality and safety of foods, providing good quality and safe products on a consistent basis is a scientific and technical challenge.

To achieve food quality and/or safety objectives, industry experts, academia, government, and regulatory agencies have worked in unison (as knowledge circles) to evolve standards, regulations, and scientific methodologies. The most challenging task is building safety in the finished products. Years of experience now suggest that consistently producing zero defect, zero pathogen products is not an achievable goal in a commercial setup. However, it is certainly possible to achieve exposure of hazards to such low levels that there is reasonable certainty of no harm to community. This in other words means deciding realistic food safety objectives. However, addressing food safety objectives needs considerable

Table 22.1. Key Provisions Insuring Purity, Wholesomeness, Balanced Nutrition, and Safety of Dairy Foods in the United States

Provision	Basis/Agency Recommendation
Standards of identity	See Table 22.2
Food labeling	21 CFR: 1B: Part 101
Nutritional quality guidelines for foods	21 CFR: 1B: Part 104
Current good manufacturing practices (CGMP) in manufacturing, packing, or handling food for human consumption	21 CFR: 1B: Part 110
Pasteurized milk ordinance (PMO)	The U.S. Department of Health and Human Services, Public Health Services and FDA

Compiled from various sources.

expertise and technical support. Toward this end of addressing food safety and quality needs of the dairy industry, the government has instilled various scientific and technical measures such as the current good manufacturing practices (CGMP), pasteurized milk ordinance (PMO), and grade A hazard analysis critical control point system (HACCP). These provisions are the results of years of profound knowledge, proactive and pragmatic enforcement activity, and practical wisdom shared among government, regulatory, scientific industry, and consumer fora (FDA, 2005). Nevertheless, onus of administrative and executive implementation has come from the government agencies proactive, yet with objective and responsible discretion. Current codes of practices and standards are not only enforceable (mechanism and infrastructure in place to properly enforce) by regulatory agencies but they are also achievable (practical) by the industry. Key provisions insuring purity, wholesomeness, balanced nutrition, and safety of milk and milk products are presented in Tables 22.1–22.3.

It is evident from the inspection and regulatory framework mechanisms that current provisions are seeking to address fundamental principles of food ethics to encourage production of safe, pure, nutritious, and wholesome food. Food industry has important responsibility to fulfil its professional food

Table 22.2. Standards of Identity for Milk and Milk Products

Product/Process	Legal Basis
Pasteurization	21 CFR 58.334
Whey	21 CFR 58.2601
Dry buttermilk and dry buttermilk product	21 CFR 58.2651
Definitions—cream pasteurized, and ultra-pasteurized	21 CFR 131.3
Milk	21 CFR 131.110
Acidified milk	21 CFR 131.111
Cultured milk	21 CFR 131.112
Concentrated milk	21 CFR 131.115
Sweetened condensed milk	21 CFR 131.120
Low-fat dry milk	21 CFR 131.123
Nonfat dry milk	21 CFR 131.125
Nonfat dry milk fortified with vitamins A and D	21 CFR 131.127
Dry whole milk	21 CFR 131.147
Dry cream	21 CFR 131.149
Heavy cream	21 CFR 131.150
Light cream	21 CFR 131.155
Light whipping cream	21 CFR 131.157
Sour cream	21 CFR 131.160
Acidified sour cream	21 CFR 131.162
Half-and-half	21 CFR 131.180
Yogurt	21 CFR 131.200
Low-fat yogurt	21 CFR 131.203
Nonfat yogurt	21 CFR 131.206
Cottage cheese	21 CFR 133.128
Dry curd cottage cheese	21 CFR 133.129
Boiler water additives	21 CFR 173.310
Whey	21 CFR 184.1979
Concentrated whey	21 CFR 184.1979(2)
Dried or dry whey	21 CFR 184.1979(3)
Reduced lactose whey	21 CFR 184.1979a
Reduced minerals whey	21 CFR 184.1979b
Whey protein concentrate	21 CFR 184.1979c

Based on Federal Food, Drug and Cosmetic Act, as amended Sec. 402 (342) Adulterated Food and Sec. 403 (343) Misbranded Food.

Table 22.3. Typical Inspection Schedule

Enforcement Action	Frequency
Dairy farm/Transfer station inspection	Every 6 month or more frequent
Milk plant/Receiving station inspection	Every 3 month or more frequent
Milk tank truck inspection	Every 12 month or more frequent
Bulk milk hauler/sampler's or industry plant sampler's pick up and sampling procedures inspection	Every 24 month
Milk tank truck cleaning facility inspection	Every 6 month or more frequent

Compiled from various sources.

ethics obligations. The main emphasis of this chapter is to provide core knowledge concerning essential elements of food safety and quality management relevant to the dairy processing industry.

CORPORATE BUSINESS CULTURE, MARKET ECONOMY, AND QUALITY MOVEMENT

Several dynamic forces such as consumerism, regulatory climate, and peer pressure from industry, academia, and professional associations have shaped food safety and quality environment of today. Knowledge circles encompassing all the stakeholders in the food sector have a collective responsibility to deliver scientific technical, and management solutions to help design and deliver consistently safe products. Such exercise could be termed as building and delivering safety quality in the finished products, from farm to the fork. Nevertheless, quality ideology has also evolved in its own right as a business tool to improve efficiency and to achieve bottom line. Quality era statisticians played a pioneering role in building and shaping quality doctrine of today (see also Deming's quality principle, Kaizen, and Six Sigma).

General Agreement in International Trade and Tariff (GATT) and market-based economy have created interesting opportunities as well as unique challenges. Food and dairy industry is no exception to this. Food commodities are traded much more than ever before. Multinational food companies operating across nations and doing global trade have made our

world a global market place. One of the important by products of market-based economy is that it could enhance fair and healthy competition and reduce unfair technical barriers. As a result, achieving consumer satisfaction through enhanced quality and service delivery should become paramount. In the corporate culture, sound quality and value-added service delivery have become hardcore business principles. The approach is not to *inspect* quality but to consistently and continuously strive to *build* quality. Today's companies are aggressively striving to integrate brand image and service delivery to compete for consumer satisfaction and customer loyalty in the market place. This is a win-win situation for the consumer as well as the manufacturer. Integration of a quality structure (management) and food safety objective (technical) could effectively address food safety expectations of consumers. As we shall see in the later part of this chapter that provisions such as ISO, TQM, and Codex standards could be easily integrated with food safety systems (Table 22.4) to enhance desirable outcome, that is, achieving processing safety objective.

As it is true with any project or model operation, meaningful participation of employees and available expertise is a key to achieving food safety and quality objectives unique to every individual setup. The take-home message here is: Market economy and quality policy has created right environment to pursue technical (food safety) goals. It is imperative that the success of the food industry in achieving food safety goals would depend upon management commitment and learning curve exhibited by cross-functional teams representing adequate expertise (including consultants, subject matter experts, third-party auditors, and mentoring from regulatory or state resource; FDA, 2004a).

CURRENT GOOD MANUFACTURING PRACTICES

CGMP ideology has its origin in federal food and drug legislations and regulatory framework development in the United States (Table 22.5).

Presence of harmful substance(s) in food is considered adulteration and it is punishable by civil and/or criminal penalties (depending upon intent, liability, negligence, and reasonable effort aspects adjudicated by case law). Good manufacturing practices guidelines aims to provide basic framework required to produce hygienic foods for human consumption. GMP has served as a model food code not only for

Table 22.4. Harmonization Between Food Safety System and Quality Management System

Food Safety System Development	Project Management Business Cycle
Hazard analysis	Market intelligence Leadership Problem identificatio Quality objectives Business process review
Determine critical control points	Process design Strategic plans Business targets Process inputs Resource allocation
Establish critical limits	Cross-functional team selection Thrust areas Cost-effective strategies Assigning responsibility Policy, procedures, SOPs Process benchmarks/targets
Establish monitoring procedures	Targeted resources Infrastructure and logistics Quality system monitoring Inspection and testing Process control Data and information generation
Establish corrective actions	Process measurements Data analysis and benchmarking Expected outcomes or corrective actions for setbacks Contingency plans/Re-prioritizing focus areas Short-term review Revised targets within available time, resource, and avenues
Establish verificatio procedures	Management review Inspection and testing Internal quality audit
Record keeping, documentation, and active review	Continuous improvement Customer-related process review Nonconformance review Corrective and preventive action review Product realization process review Corporate feedback

Compiled from various sources.

the food operations in the United States, but also for the rest of the world in its one form or another. GMP has continued to evolve to reflec current needs and proactive responsible governance. Presently, GMP is undergoing revision to reflec needs of the twenty-firs century.

PRINCIPLES AND ESSENTIAL ELEMENTS

GMP encompasses following areas (Table 22.6) considered important for producing clean and hygienic food for human consumption.

Table 22.5. GMP Milestones

Time	Milestone Event
1906	The Bureau of Chemistry passes the 1906 Pure Food and Drugs Act addressing misbranding and adulteration concerns
1933	FDA recommends revision of Pure Food and Drugs Act
1938	FDA passes Federal Food, Drugs, and Cosmetics Act. Product Identity and quality standards for food introduced
Mid-1960s	FDA decides to formulate GMP regulations to help implement food law
1969	Food GMP regulations finalize
Early 1970s	Emphasis on industry-specific regulations
Late 1970s	Focus back to general GMPs rather than industry-specific
1986	Revised food GMPs published
2004	Modernization of GMPs for twenty-first century being developed

Source: FDA (1981) and Dunkelberger (1995).

These provisions reflect profound knowledge and years of scientific and anecdotal experience in food safety. As a continuous exercise to modernize GMPs and to enhance their effectiveness and relevance, a comprehensive revision is being undertaken by the Federal Food and Drug Administration (FDA) and associated agencies. In the revised version, it is recognized that realities of ready-to-eat (RTE) segment needs to be addressed adequately. Consumers now prefer RTE fresh salads outside instead of salads prepared at home (FDA, 2004b). Consumers also increasingly opt for foods that need no cooking/minimal cooking (e.g., microwavable foods)

before use. This requires greater than usual care in controlling foodborne hazards during manufacturing and storage of foods. There is now increased understanding and better risk analysis data available for principal food safety concerns such as *Listeria monocytogenes* (LM), *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Salmonella enteritidis*, *Cryptosporidium parvum*, Noroviruses, food allergens, and chemical contaminants, and so forth. To address these concerns, modernized CGMP would seek to emphasize following focus areas.

Table 22.6. Essential Elements of Current GMP Legislation

21 CFR Part 110	Focus Area
Section 110.3	Definition
Section 110.5	Current good manufacturing practice
Section 110.10	Personnel
Section 110.19	Exclusions
Section 110.20	Plants and ground
Section 110.35	Sanitary operations
Section 110.20	Sanitary facilities and controls
Section 110.40	Equipment and utensils
Section 110.80	Processes and controls
Section 110.93	Warehousing and distribution

Source: Federal Register (1986).

1. A risk-based approach requiring integration of regulatory requirements with food safety outcomes.
2. Appropriate training. Experience has taught us that without adequate training of supervisors and floor workers, improvement is minimal. Specific training with regard to importance of hygiene and sanitation (personal as well as premises, surroundings and operational territories) in food protection and producing safe food is essential to make impactful change.
3. Food allergen control plan. In view of numerous recalls associated with allergens and likely danger of allergen exposure to sensitive population, new regulations would require food allergen control plan by the manufacturers. Under this requirement, the manufacturer must address six elements: (i) training of processing and supervisory personnel, (ii) segregation of food allergens during storage and handling, (iii) validated cleaning

- procedures for food contact equipment, (iv) prevention of cross-contact during processing, (v) product label review and label usage and control, and (vi) a supplier control program for ingredients and labels.
4. Written environmental pathogen control program for production of RTE foods that support growth of LM. LM is difficult to control in the plant environment and it is necessary to microbiologically monitor the food processing environment as both validation of current cleaning and sanitizing procedures and as a verification procedure to identify LM sources in the food plant environment.
 5. Written sanitation procedures. It is realized that sanitation is a key to minimizing postprocessing contamination and biofilm build up. However, sanitation is often less than adequate and unorganized area of operation/or priority. To address this, revised CGMP would require that food processors develop and maintain written sanitation procedures that define the scope, sanitation objective, management responsibility, monitoring, corrective action, and record keeping associated with the sanitation procedure.
 6. Maintenance of critical records. Food processors would be required to maintain critical records that should be made available for review and evaluation. Critical records are those records that a processor (or FDA) would need to review in order to confirm that a firm is operating in compliance with the CGMP regulation. This is consistent with well known quality system principle that control procedures must be defined, documented, reviewed, and appropriate corrective action taken.

According to FDA, modernized CGMP would facilitate control of food safety hazards for food manufacturers by logical allocation of resources with maximum impact on food safety. Improved measures suggested in the amended regulation are based on risk analysis and they have been proven to significantly reduce the risk of foodborne hazards. Some of the other salient features of CGMP are summarized as below.

- Relatively straightforward structure. Easy to understand and implement.
- Forms the basis of foundational platform for Quality Assurance program (QA) upon which other systems such as food safety system (e.g., HACCP) could be built.
- Well-done GMPs could serve as prerequisite programs (PP) on which effective HACCP system could be built.

- Intelligently laid out, company specific GMP system could provide safety net and cost savings with beneficial influence for the secondary system (HACCP then could be directed to focus essential processing control).

Some of the probable pitfalls associated with GMP could be:

- It is not very specific and detail oriented. There is room for subjective interpretation and discretion as to what are acceptable versus borderline situations.
- It may not be possible to implement all the provisions at a time for smaller or mid-sized companies due to lack of resources and necessary expertise.
- Less amenable to knowledge exchange and information sharing to compare specific QA plans.
- Relative absence of benchmarks or reference standards to facilitate objective internal performance review (Blame game!).

DAIRY FOOD SAFETY SYSTEMS

HACCP- and PMO-based systems serve to address dairy food safety-related requirements for the dairy industry. National Conference on Interstate Milk Shipments (NCIMS) is promoting dairy HACCP (grade A voluntary HACCP) to enhance food safety in dairy processing operations. However, PMO-based dairy processing safety system would remain a fundamental force upon which other systems could be built in unison or developed as distinct programs. Experience has suggested that stand-alone HACCP programs are not likely to be successful or effective. On the other hand, detailed specification and framework laid out in PMO could provide reliable, validated, and time-tested logistics and procedures to build strong HACCP program.

According to Howard Bauman, HACCP is a preventive system of control based on rational and logical process of estimating the risk associated with production and marketing a given food product. Control of food processing could be obtained and maintained through diligent, intelligent application of the principles of hazard analysis and the identification of control points that are critical to food safety (Bauman, 1974, 1995). According to advisory committee on microbiological criteria, HACCP is a management system in which food safety is addressed through the analysis and control of biological,



Figure 22.1. Howard Bauman is considered father of HACCP for his pioneering work at Pillsbury Co. (Photo courtesy: Institute of Food Technologists (IFT), Chicago, IL.)

chemical, and physical hazards from raw material production to procurement, handling, manufacturing, distribution, and consumption of the finished product. HACCP is a common-sense, practical, and achievable food safety approach that industry is striving to achieve within the limitations of the available technology to produce, transport, procure, and prepare foods that present a minimum level of risk from foodborne hazards (Bauman, 1974). Mossel et al. (1997, 1998) have defined HACCP as a hazard analysis (carried out to achieve) control of critical practices.

Howard Bauman, then VP of Science and Technology, Pillsbury Company, Minnesota, is considered “father of HACCP” due to his pioneering work (Fig. 22.1) in developing and successfully implementing HACCP concept for production of “zero defect” foods for National Aeronautics and Space Administration (NASA) space program.

The ideology of HACCP is the result of team effort by the Pillsbury Company, NASA, and the Army Natick Laboratory to apply a zero-defect program to food production. Their system was based on Failure Mode Effect Analysis (FMEA) and risk analysis tools. It is possible that genesis of HACCP has its roots in quality management and process control

ideology of Deming and other quality pioneers who developed process control tools such as FMEA, Six Sigma, and trend analysis. However, Dr. Bauman is credited not only for visualizing (creativity, innovation!) HACCP model but also, successfully integrating it with quality management tools and food safety outcomes. Since then, HACCP concept has continued to evolve (Table 22.7, HACCP chronology).

Practicality of HACCP concept and associated cost-benefit has spurred tremendous interest in this model throughout the world (Fig. 22.2).

The U.S. Department of Agriculture (USDA) and the FDA have embraced HACCP as an effective food safety tool (Table 22.7).

The influence of HACCP ideology has such a profound impact on national food safety goals that FDA has instituted an annual Bauman Award to felicitate individual(s) or organization(s) who make significant contribution to improving food safety.

While dairy HACCP is relatively recent to the dairy processing industry, PMO has been around for a while. Fundamental basis of PMO has been to enhance uniformity and higher level of milk sanitation excellence in the United States. It encourages legal adaptation by states, counties, and municipalities to facilitate shipment and acceptance of milk and milk products of high sanitary quality in interstate and intra-state commerce (FDA, 2003a). This ordinance has been widely adapted and is in use for many years now and has been upheld by court decisions. Key provisions underlying PMO and related standards are given in Table 22.8. For more detailed information, readers are referred to PMO document available online at <http://www.cfsan.fda.gov/~ear/pmo03toc.html>.

It is pertinent to note here that one of the most stimulating features of PMO document is its *Public Health Reason* explanatory notes, wherein scientific rationale and years of practical knowledge is presented with accuracy and clarity. The document is quite user-friendly and could serve as training resource by itself.

In this chapter, more emphasis is given to HACCP because it is relatively recent in the dairy industry and there is a critical need for more information on this topic. On the contrary, PMO system is well worked out in terms of implementation needs (by industry) and enforcement dynamics (issues and situations in enforcement). With this presumption, National Conference on Interstate Milk Shipments (NCIMS, 2006) grade A dairy HACCP would be discussed in more details to provide up-to-date information on this challenging, yet exciting food safety concept.

Table 22.7. HACCP Chronology

1971	Discussions started at the National Conference on Food Protection. Risk assessment combined with the critical point concept.
1970s	Pillsbury firs used HACCP for safety.
1985	National Academy of Science recommended HACCP for broad categories of foods.
1989	The U.S. National Advisory Committee on Microbiological Criteria for Food (NACMCF) developed updated HACCP system. Federal agencies responsible for food safety endorsed HACCP-based food safety system.
1990s	HACCP started gaining international acceptance.
1997	Seafood HAACP program becomes mandatory.
1998	Meat and poultry HACCP becomes mandatory for large manufacturers.
1999	Meat and poultry HACCP becomes mandatory for small manufacturers.
1999	Pilot HACCP program for National Conference of Interstate Milk Shipment (Dairy HACCP).
2002	Mandatory juice HACCP for processors and small businesses.

Source: Ohio State University Extension Bulletin 901.

IMPLEMENTING HACCP (WHERE RUBBER MEETS THE ROAD!)

HACCP implementation is a systematic exercise of identifying, evaluating, and controlling food safety hazards based on the following essential principles (FDA, 1997).

Principle 1: Conduct a hazard analysis.

Principle 2: Determine the critical control points (CCPs).

Principle 3: Establish critical limits.

Principle 4: Establish monitoring procedures.

Principle 5: Establish corrective actions.

Principle 6: Establish verificatio procedures.

Principle 7: Establish record-keeping and documenta-
tion procedures.

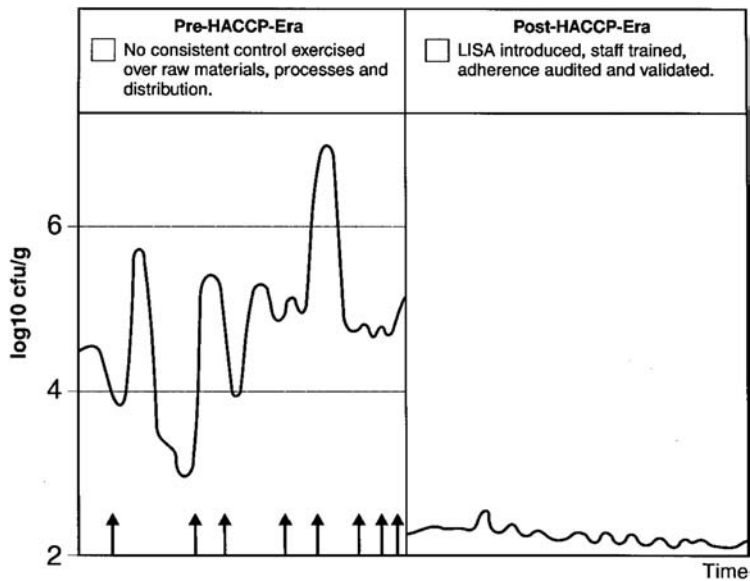


Figure 22.2. Comparison of Pre-HACCP and post-HACCP era food safety performance matrix as a function of microbial load in the finished product. (Courtesy: Mossel et al. Int. J. Environmental Health Research, 7:233 (1997).)

Table 22.8. PMO Standards for Milk and Milk Products

Grade “A” raw milk and milk products for pasteurization, ultra-pasteurization, or aseptic processing	Temperature	Cooled to 10°C (50°F) or less within 4 hours or less, of the commencement of the first milking, and to 7°C (45°F) or less within 2 hours after the completion of milking. Provided, that the blend temperature after the first milking and subsequent milkings does not exceed 10°C (50°F).
	Bacterial limits	Individual producer milk not to exceed 100,000 per mL prior to commingling with other producer milk. Not to exceed 300,000 per mL as commingled milk prior to pasteurization.
	Drugs	No positive results on drug residue detection methods as referenced in Section 6—Laboratory.
	Somatic cell count ^a	Individual producer milk not to exceed 750,000 per mL.
	Temperature	Cooled to 7°C (45°F) or less and maintained thereafter.
	Bacterial limits ^b	20,000 per mL, or gram. ^c
	Coliform ^d	Not to exceed 10 per mL. Provided, that in the case of bulk milk transport tank shipments, shall not exceed 100 per mL.
	Phosphatase ^e	Less than 350 milliunits/L for fluid products and other milk products by the Fluorometer or Charm ALP or equivalent.
	Drugs ^b	No positive results on drug residue detection methods as referenced in Section 6—Laboratory Techniques which have been found to be acceptable for use with pasteurized and heat-treated milk and milk products.
	Temperature	Cooled to 7°C (45°F) or less and maintained there at unless drying is commenced immediately after condensing.
Grade “A” pasteurized concentrated (condensed) milk and milk products	Coliform	Not to exceed 10 per gram. <i>Provided</i> , that in the case of bulk milk transport tank shipments shall not exceed 100 per mL.
	Temperature	None.
	Bacterial limits	Refer to 21 CFR 113.3(e)(1) ^f
	Drugs ^b	No positive results on drug residue detection methods as referenced in Section 6—Laboratory Techniques that have been found to be acceptable for use with aseptically processed milk and milk products.

(cont.)

Table 22.8. (cont.)

Grade “A” whey for condensing	Grade “A” nonfat dry milk		No more than:	
	Butterfat		1.25%	
	Moisture		4.00%	
	Titratable acidity		0.15%	
	Solubility index		1.25 mL	
	Bacterial estimate		30,000 per gram	
	Coliform		10 per gram	
	Scorched Particles disc B		15.0 per gram	
	Temperature		Maintained at a temperature of 45°F (7°C) or less, or 63°C (145°F) or greater, except for acid-type whey with a titratable acidity of 0.40% or above, or a pH of 4.6 or below.	
			Cooled to 7°C (45°F) or less during crystallization, within 48 hours of condensing.	
Grade “A” pasteurized condensed whey and whey products	Temperature		Not to exceed 10 per gram	
	Coliform limit		Not to exceed 10 per gram	
Grade “A” dry whey, grade “A” dry whey products, grade “A” dry buttermilk, and grade “A” dry buttermilk products	Coliform limit			
	Coliform limit			

Source: FDA (2003a).

^a Goat Milk 1,000,000 per mL.

^b Not applicable to acidified or cultured products.

^c Results of the analysis of dairy products which are weighed in order to be analyzed will be reported in # per gram.

^d Not applicable to bulk shipped heat-treated milk products.

^e Not applicable to bulk shipped heat-treated milk products; UP products that have been thermally processed at or above 138°C (280°F) for at least 2 seconds to produce a product which has an extended shelf life (ESL) under refrigerated conditions and condensed products.

^f 21 CFR 113.3(e)(1) contains the definition of “COMMERCIAL STERILITY.”

For HACCP implementation to be successful, certain prerequisite programs (PP) should be in place. CGMP, PMO, and Food Code are good guides for implementing PP. According to FDA, important characteristics of well-done prerequisite programs are:

- Sanitary establishments and facilities with linear product flow and logical traffic pattern minimizing cross-contamination from raw to processed products.
- Well-executed supplier assurance and verification program.
- Clear and regularly updated ingredient, product and packaging material specification reflecting known concerns.
- Sanitary processing equipment with preventive maintenance and regular calibration schedules.
- Written cleaning and sanitation procedures with aggressive verification schedule.
- Adequate employee hygiene and training. Finding out problem areas and continuously innovating to address this important issue.
- Pest control and nonfood chemical hazard control.
- Well-rehearsed recall and traceability procedures.
- Well laid out standard operating procedures (SOPs) for processes, product formulations, sanitation, and allergen control.
- Proactive glass and metal control programs.
- Well laid out shipping, receiving, and storage procedures and logistics.

While prerequisite programs may impact HACCP, these programs are also designed to ensure wholesomeness and suitability for food destined for human consumption. In contrast, HACCP plans are narrower in scope and specific to ensuring food safety. Nevertheless, existence and effectiveness of PP should be assessed afresh during the design and implementation of each HACCP plan module. All PP should be documented and regularly audited. To adequately address integration of PP with HACCP, NCIMS emphasizes substantial compliance as to whether:

- ☐ A. Required prerequisite program (PP) written, implemented, and in substantial compliance by firm
 - ☐ 1. Safety of the water that comes into contact with food or food contact surfaces (including steam and ice).
 - ☐ 2. Condition and cleanliness of equipment food contact surface.
 - ☐ 3. Prevention of cross-contamination from unsanitary objects and/or practices to

food products, packaging material and other food contact surfaces, including utensils, gloves, outer garments, and so forth, and from raw product to processed product.

- ☐ 4. Maintenance of hand washing, hand sanitizing, and toilet facilities.
- ☐ 5. Protection of food, food packaging material, and food contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate, and other chemical, physical, and biological contaminants.
- ☐ 6. Proper labeling, storage, and use of toxic compounds.
- ☐ 7. Control of employee health conditions that could result in the microbiological contamination of food, food packaging materials, and food contact surfaces.
- ☐ 8. Pest exclusion from the food plant.
- ☐ B. Additional PPs required or justified by the hazard analysis are written and implemented by firm
- ☐ C. PP conditions and practices monitored as required.
- ☐ D. PP monitoring performed at a frequency to ensure conformance.
- ☐ E. Corrections performed in a timely manner when PP monitoring records reflect deficiencies or nonconformities.
- ☐ F. PP audited by firm
- ☐ G. PP monitoring records adequately reflect conditions observed.
- ☐ H. Prerequisite program signed and dated as required.

GET ORGANIZED

Before applying seven principles of HACCP, following preliminary steps are imperative (FDA, 2004c).

HACCP being a scientific doctrine, systematic and organized work plan is required as would be expected in any scientific endeavor. Isn't one simple definition of science systematic and methodical (organized) effort at problem solving?

HACCP TEAM (WHERE IS MY DREAM TEAM?)

HACCP team should adequately represent available expertise and knowledge base representing product and process areas. Typically, cross-functional team

comprising food microbiology, quality assurance, engineering, production, and sanitation department is ideal. Outside experts, consultants, and specialists could be involved in designing and validating HACCP projects depending upon needs. A generic HACCP (one model fit all) is not possible in the food industry due to uniqueness and differing complexities in each food operation. Therefore, a thorough analysis about food manufacturing cycle from cow to consumer should factor-in all the vulnerable points and likely logistical/consumer abuse associated with each operation. In other words, careful risk analysis and probable failures considered in developing food safety objective, in essence, define and differentiates each HACCP plan, and so does its success or failure. The bottom line here is: Seeking outside help and expert consultation, depending upon unique needs, would be cost-effective and highly beneficial in objective analysis of HACCP design and implementation.

DESCRIBE YOUR FOOD, ITS DISTRIBUTION DYNAMIC, AND INTENDED USE BY THE CONSUMERS

Adequate description of food (ingredients, processing methods), its distribution method (frozen, refrigerated or ambient), and the end user (general public or a segment viz. infant, elderly, or immunocompromised) helps adequately integrate risk factors with food safety goals (Buchanan and Whiting, 1998). For example, diabetic ice cream or infant food has a much lower threshold for LM because of vulnerability of this segment of population. In the United States, qualitative risk analysis already factors into such sensitive population in determining risk exposure. However, pertinent details about nature of food, cold chain logistics, and consumer usage matrix are important variables that need to be plugged into HACCP plan development.

For NCIMS dairy HACCP, typical product description examples are shown in Tables 22.9 and 22.10. These examples adequately represent product characteristic, distribution pattern, and expected end use relevant for HACCP plan development.

DEVELOP A FLOW DIAGRAM AND PHYSICALLY VERIFY THAT THE FLOW DIAGRAM IS CURRENT AND ACCURATE

Flow diagram should include all the steps occurring from reception up until distribution under direct con-

Table 22.9. Product Description Example 1

General product description	Pasteurized and homogenized fluid whole milk fortified with vitamin A. Milk is filled in 1 gallon plastic bottles with tamper evident seal
Distribution and storage	Stored and distributed under refrigerated conditions (<45°F)
Intended use and consumers of the food	Intended for retail sale as ready-to-drink product for all

Source: FDA (2007f).

trol of the manufacturing establishment. The flow diagram does not need to be as complex as engineering drawing. A simple schematic would serve the purpose. However, accuracy and completeness of flow diagram is important. HACCP team should physically verify the flow diagram and any modification or corrections should be documented.

HAZARD ANALYSIS

Hazard analysis exercise involves two interrelated steps: (1) hazard identification (stage I) and (2) critical evaluation of identified hazards (stage II). The main purpose of hazard analysis is to develop a list of hazards that are of such significance that they are reasonably likely (pose sufficient risk) to cause injury/illness, if not effectively addressed (absence of instituting active control).

Hazard identification involves review of all the possible hazards (physical, chemical, or biological) gleaned from scientific/technical literature, government resources, company records, product recall/failure logs, consumer complaint records, and other knowledge sources. It is important to consider sensitive ingredients, raw materials, processes, storage, and distribution steps, and final preparation and end-use by consumers as part of developing a risk model. In Tables 22.11–22.13, relevant information about pathogen risk associated with cheese manufacturing is presented. It would appear from this data that food safety risk pertaining to pathogens; toxins, antibiotics, and extraneous matter need to be effectively addressed. Absence of control measures would otherwise pose significant risk.

Table 22.10. Product Description Example 2

Formal Product Name	Reduced Fat Milk (2%)
Product description and food safety characteristics	Pasteurized and homogenized fluid 2% milk fortified with vitamin A. Milk is filled in 1 gallon plastic bottles with tamper evident seal. Support growth of a number of pathogens. No natural protective characteristics
Packaging used	High density polyethylene gallon container with a polypropylene snap-on screw-off tamper evident cap. Labels are self-adhesive and applied prior to filling. Code date is printed via coding equipment after capping
Distribution and storage	Product is cased in standard milk cases—four units per case. Temperature of storage is $\leq 45^{\circ}\text{F}$. Distributed using refrigerated trucks ($\leq 45^{\circ}\text{F}$) to wholesale and retail outlets
Labeling requirements	Keep refrigerated, Grade “A,” pasteurized, homogenized, vitamins A and D added, 30% less fat than regular milk
Intended consumers of the food	Consumers of all ages consume this product
Intended use	Ready to serve product. May also be used as an ingredient in preparing meals
Shelf life	16 days under proper refrigeration

Source: FDA (2007f).

Following aspects should be considered during hazard analysis exercise:

1. Sensitivity of ingredients. Do ingredients contain microbial (LM, EC, SA), chemical (Aflatoxin antibiotics), or physical (glass, metal, stone) hazards? Is geographical location of supplier critical?
2. Intrinsic food factors. Does food composition by itself influence hazard? Does food permit survival or multiplication of pathogens during production, storage and/or distribution? What is known about safety record of similar products in the market? What hazards have been known for similar products?
3. Is processing adequate to destroy pathogens? Is postprocessing contamination likely? Which specific hazards would be likely?
4. What other safety devices (magnets, metal detectors, sifters, filters thermometers) are in place to enhance consumer safety?
5. Storage logistics. What is the temperature profile of cold chain? Are there unacceptable variations and frequencies? Cold temperature abuse result into growth of pathogen(s)?
6. Consumer mishandling/abuse. Is consumer likely to heat the product? Are there left-over expected? Is cross-contamination or storage abuse due to lack of instruction expected?

To emphasize criticality of above aspects during hazard analysis stage, NCIMS dairy HACCP aims to verify specifically whether:

- ☐ A. Flow diagram and hazard analysis conducted and written for each kind of group of milk or milk product processed.
- ☐ B. Written hazard analysis identifies all potential food safety hazards and determines those that are reasonably likely to occur (including hazards within and outside the processing plant environment).
- ☐ C. Written hazard analysis reassessed after changes in raw materials, formulations, processing methods/systems, distribution, intended use, or consumers.
- ☐ D. Written hazard analysis signed and dated as required.

After potential hazards are identified hazard evaluation step ensues. Hazard evaluation is akin to brainstorming. Basically, at this stage determination is made on the basis of scientific technical, and objective analysis as to whether identify hazard(s) are reasonably likely to cause illness or injury in the absence of controls. This step is comparable to failure mode effect analysis (Table 22.14) and risk assessment exercises (Fig. 22.3).

Table 22.11. Various Biological, Chemical, and Physical Hazards Associated with Dairy Products

Fluid Milk	Cheese	Ice Cream	Dried Milk	Whey	Condensed Products
Biological	Biological	Biological	Biological	Biological	Biological
<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>	<i>Salmonella</i>	<i>L. monocytogenes</i>	<i>Salmonella</i>
<i>L. monocytogenes</i>	<i>L. monocytogenes</i>	Mold Spores	<i>L. monocytogenes</i>		<i>L. monocytogenes</i>
<i>S. aureus</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>		<i>S. aureus</i>
<i>S. enterotoxin</i>	<i>S. enterotoxin</i>	<i>E. Coli</i>	<i>S. enterotoxin</i>	<i>E. coli</i>	<i>S. enterotoxin</i>
<i>C. perfringens</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. perfringens</i>
<i>E. coli</i>	<i>Campylobacter</i>	Chemical	<i>C. botulinum</i>	<i>S. enterotoxin</i>	<i>Yersinia</i>
<i>Yersinia</i>	Soil	Non-Food	<i>C. perfringens</i>		<i>Campylobacter</i>
<i>enterocolitica</i>	Glass fragments	Chemical Vapors	Chemical		<i>B. cereus</i>
<i>Campylobacter</i>	Wood splinters		Sulfonamides		<i>Shigella</i>
<i>B. cereus</i>	Metal fragments		Antibiotics		<i>Brucella</i>
<i>Shigella</i>			Pesticides		Chemical
<i>Brucella</i>	Nitrates, nitrites				Antibiotics
	Aflatoxi				Pesticides

Reproduced with permission from Gould et al. (2000), The Economics of HACCP, Eagan Press, St. Paul, MN.

Table 22.12. Selected Foodborne Outbreaks Associated with Cheese Reported from Various Parts of the World

Year	Country	Pathogen	#of Cases	#of Deaths	Cheese	Description
1983	Netherlands, Denmark, Sweden, USA	Enterotoxigenic <i>E. coli</i>	> 3,000	NR	Brie	Use of raw milk
1983–87	Switzerland	<i>L. monocytogenes</i>	> 122	34	VacherinMont d'Or	Made from thermalized milk
1984	Canada	<i>Salmonella typhimurium</i>	2,700	1	Cheddar	<i>Salmonella</i> survived up to 8 months in refrigeration
1984–85	Scotland	<i>Staphylococcus aureus</i> enterotoxin	> 13	0	Sheep milk	Mastitis and post-infection carriage by ewes
1985	USA	<i>L. monocytogenes</i>	> 142	48	Mexican style	Pigsty contamination caused by addition of raw milk
1989	England	<i>Salmonella dublin</i>	42	0	Irish soft	Four cows were asymmetric excretors, <i>Salmonella</i> detected in curd but not in raw milk or factory

Reproduced with permission from Gould et al. (2000), The Economics of HACCP, Eagan Press, St. Paul, MN.
NR, not reported.

Table 22.13. Selected Foodborne Outbreaks Associated with Cheese Reported from Various Parts of the World

Year	Country	Pathogen	#of Cases	#of Deaths	Cheese	Description
1989	USA	<i>Salmonella javiana</i> and <i>S. oranienberg</i>	164	0	Mozzarella	Contamination: 0.36–4.3 cells/100 g
1992–93	France	Verocytotoxin forming <i>E. coli</i>	NR	1	Fromage Frais	
1993	France	<i>Salmonella paratyphi B</i>	273	1	Goats milk	Micro, monitoring failed to detect for 2 months
1994	Scotland	Verocytotoxin forming <i>E. coli</i>	>20	0	Local farm produced	
1995	France	<i>L. monocytogenes</i>	20	4	Brie de Meaux	Disinfection and control measures reinforced
1998	Canada	<i>Salmonella typhimurium</i>	>650	0	Cheddar, Swiss	Found in Lunchmates, cross contamination possible

Reproduced with permission from Gould et al. (2000), The Economics of HACCP, Eagan Press, St. Paul, MN.
 NR, not reported.

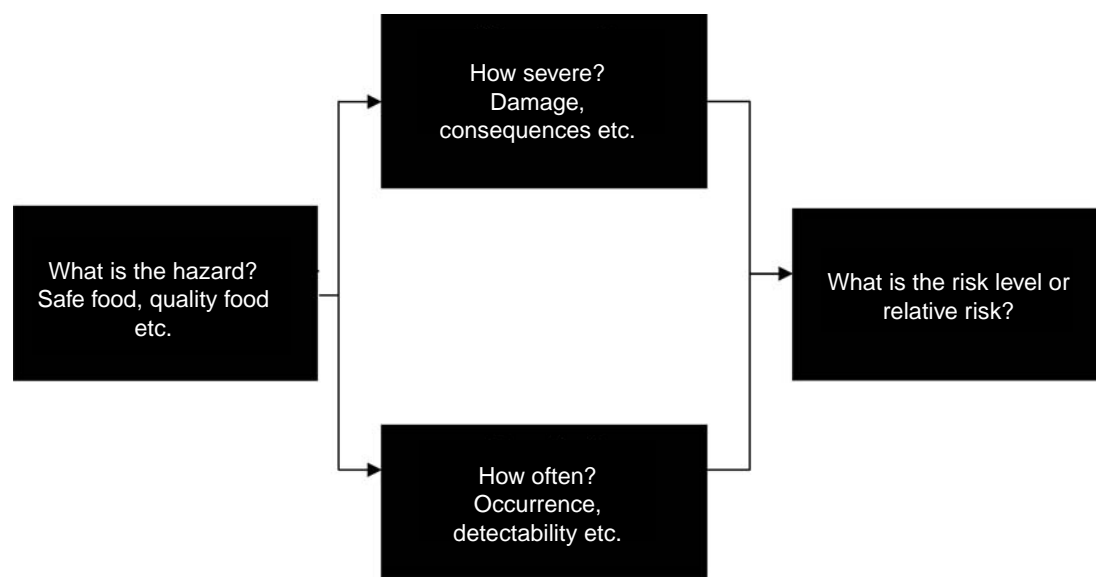


Figure 22.3. Risk analysis matrix seeks to ask four simple but related questions.

In a typical example of cake mix, poor quality cake (hazard) is attributable to human element and instrumental shortcomings. Severity, occurrence, and detectability of such hazard constitute relative risk (ranging from 75 to 225 in above example). After implementing recommended actions (use of large-print measuring cups, replacing faded cups, imparting training, and developing standard operating procedures), milk cake failure rate is reduced significantly (with risk levels ranging from 5, 10, 24, and up to 75). Another example of hazard evaluation (qualitative risk analysis) in a dairy processing setup is given in Table 22.15.

On the basis of scientific evidence, two hazards are identified (i) vegetative pathogens (biological) and (ii) animal drug residues (chemical). However, practical implementation suggests that later hazard (drug residues) could be adequately controlled through adulteration control. In contrast, food safety hazard associated with vegetative pathogens is reasonably likely to occur based on historical data.

Important outcome of hazard evaluation is determination of critical points in processing, that is, which aspects of processing are *critically* important in ensuring food safety that require specific attention. Hazard evaluation exercise might impact existing

manufacturing (product, process or both) practices in that certain changes might be necessary to enhance food safety. In a typical cheese processing setup, raw milk receiving, dairy ingredient storage, raw cream storage, pasteurization, culture media pasteurization, milling and metal detection steps are determined to be critical for safe cheese manufacture. Loss of control at one or more of these critical steps would pose unacceptable risk. As shown in this example, specific control steps are instituted to address significant hazards.

It would appear from above analysis that hazard evaluation is comparable to quantitative risk analysis. Presently, only qualitative risk analysis is performed in dairy processing setup. However, risk modeling and quantitative risk analysis as described below would become possible in future (see Tables 22.16–22.20; see Fig. 22.4).

Mid-sized or smaller firm lacking sufficient resources to carry out such complex risk analysis exercise could take advantage of government resources. Especially, FDA’s pathogen modeling project aims to make risk analysis and food safety evaluations widely available to its stakeholders keeping in mind the usefulness but at the same time realizing practical difficulties and complexity of such exercises.

Table 22.15. Generic Example of Hazard Analysis

SUBJECT Hazard Analysis Worksheet PLANT NAME ADDRESS		ISSUE DATE		PRODUCT	
		SUPERSEDES		PAGE	
(1) Ingredient/Processing Step	(2) Identify <i>potential</i> food safety hazards introduced, controlled or enhanced ^a at this step.	(3) Are any <i>potential</i> food-safety hazards reasonably likely to occur?	(4) Justify your decision for column 3	(5) What control measure(s) can be applied to prevent, reduce, or eliminate the food safety hazards?	(6) Is this step a critical Control point? (Yes/No)
	Biological Physical Chemical Biological Physical Chemical Biological Vegetative Pathogens Physical None Chemical Animal drug residues	<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO	 Likely to occur based on historical data (Leave Blank) Appendix N Testing in PP #5 Protection from Adulteration	 Pasteurization (Leave Blank) (Leave Blank)	<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO

^aDo not carry the hazard through subsequent steps.

Source: NCIMS Dairy HACCP web resources, FDA (2007i). Simplified for illustrative purpose of hazard analysis. For accurate legislative details, readers are referred to federal web/print resources.

Table 22.16. Typical Pathogen Exposure Modeling Illustration Based on Infective Dose of 1

One infected portion of food encountered for a population base with its typical consumption pattern	Statistical probability (<i>Q</i>)			
	0.950	0.990	0.995	0.999
	Exposure once in about			
	20 years	100 years	200 years	1,000 years
2.0×10^5	2.6×10^{-7}	5.0×10^{-8}	2.5×10^{-8}	5.0×10^{-9}
4.7×10^7	1.1×10^{-9}	2.1×10^{-10}	1.1×10^{-10}	2.1×10^{-11}
3.0×10^9	1.7×10^{-11}	3.4×10^{-12}	1.7×10^{-12}	3.3×10^{-13}
4.7×10^{10}	1.1×10^{-12}	2.1×10^{-13}	1.1×10^{-13}	2.1×10^{-14}

Source: Mossel and Drion (1979) with permission from Springer Link. Terminologies modifie for illustration purpose.

Table 22.17. Exposures Envisaged Under Various Scenarios

Scenario	Exposure as projected in Table 22.16	Typical Consumption Pattern
1	2.0×10^5	1.5% of The Netherlands' population eats one portion of a food once a year, e.g., turkey at Christmas time
2	4.7×10^7	All infants in The Netherlands take one portion per day during 270 days
3	3.0×10^9	72% of the population of The Netherlands eat one portion 300 times a year
4	4.7×10^{10}	72% of the population of the United States of America eat one portion 300 times a year

Source: Mossel and Drion (1979) with permission from Springer Link. Terminologies modifie for illustration purpose.

Stages I and II of hazard analysis lead to next step of HACCP, that is, determination of CCPs. At this stage, list of theoretically possible hazards is narrowed down to: few specific hazard (associated critical points) that may need to be considered by a product manufacturing facility.

PRINCIPLE 2: DETERMINE CRITICAL CONTROL POINTS

A CCP is defined as a step at which control could be applied and application of such control is essential to prevent or eliminate a food safety hazard or to reduce

Table 22.18. Typical Pathogen Exposure Modeling Based on Various Infective Doses and Anticipated Consumption Pattern (*Q* = 0.99)

Minimum Infective Dose Level	One Infected Portion of Food Encountered for a Population Base with Its Typical Consumption Pattern			
	Scenario 1	Scenario 2	Scenario 3	Scenario 4
1	5×10^{-8}	2.1×10^{-10}	3.4×10^{-12}	2.1×10^{-13}
10	9.2×10^{-1}	5.1×10^{-1}	3.3×10^{-1}	2.5×10^{-1}
100	5.6×10	5.0×10	4.6×10	4.4×10
1,000	8.3×10^2	8×10^2	7.9×10^2	7.8×10^2

Source: Mossel and Drion (1979) with permission from Springer Link. Terminologies modifie for illustration purpose.

Table 22.19. Risk Analysis Matrix for the Transmission of *L. monocytogenes* by Dry Milk

<i>(1) Raw milk phase</i>	
Fraction of total number of cows, supplying raw milk, which are suffering from subclinical <i>Listeria</i> mastitis	d
Mean number of cfu of <i>Listeria</i> per 1 mL intra vitam milk of shedding cows	c
Contamination with <i>Listeria</i> from environment	e
Proliferation of initial total contamination	Δ_r
<i>(2) Pasteurization stage</i>	
Reduction resulting from clarification	R_c
Contamination from pre-pasteurization area, including inadequately cleaned apparatus	ρ_p
Lethality of pasteurization process	Λ_p
Recontamination from raw milk circuit through microleaks	ρ_r
Environmental contamination after pasteurization	ρ_e
Proliferation before condensation	Δ_p
<i>(3) Drying process</i>	
Lethality of condensation	Λ_c
Lethality of spray drying	Λ_d
Lethality during storage in dried condition	Λ_e

Source: Mossel et al. (1998). Reproduced with permission from Elsevier Publishers.

Table 22.20. Risk Analysis Matrix for the Transmission of *Staphylococcus aureus* and/or Its Toxin in Cheese Manufacture Setup

<i>Event 1: post-pasteurization contamination with Staph. aureus</i>	
Total recontamination in cfu/g	r
Fraction of population being <i>Staph. aureus</i>	s
Enterotoxinogenic part of <i>Staph. aureus</i> population	e
<i>Event 2: proliferation of enterotoxinogenic strains during the various stages of manufacture and maturation (Δ)</i>	
Abuse temperature/time integral	$\int T dt$
Inhibition due to the development of <i>Lactobacteriaceae</i> resulting in acidification of the curd and the production of nitrogen-containing inhibitory metabolites	I
Growth retardation resulting from progressively anaerobic conditions	an
<i>Event 3: enterotoxin formation (τ); cf Appendix A^a</i>	
Time/temperature integral as under 2	$\int T dt$
Competition	I
Retardation	an

Integration of effects of events 1–3

Assuming consumption of a portion of about 100 g cheese and the minimum toxic dose of enterotoxins being of the order of 1 μ g, the risk of contracting staphyloenterotoxiosis as a result of the consumption of one portion of a given consignment of hard cheese, equals: $100 \cdot rse \cdot \Delta[f_1(\int T dt \cdot I \cdot an)] \cdot \tau \cdot [f_2(\int T dt \cdot I \cdot an)]$

Assessment of the risk of staphyloenterotoxins being transmitted, at a level leading to disease, by hard cheeses manufactured according to GMP including: (1) use of adequately pasteurized milk; (2) under conditions where starter culture activity was checked and found satisfactory.

^a Readers are referred to original paper for detailed discussion of the subject.

Source: Mossel et al. (1998). Reproduced with permission from Elsevier Publishers.

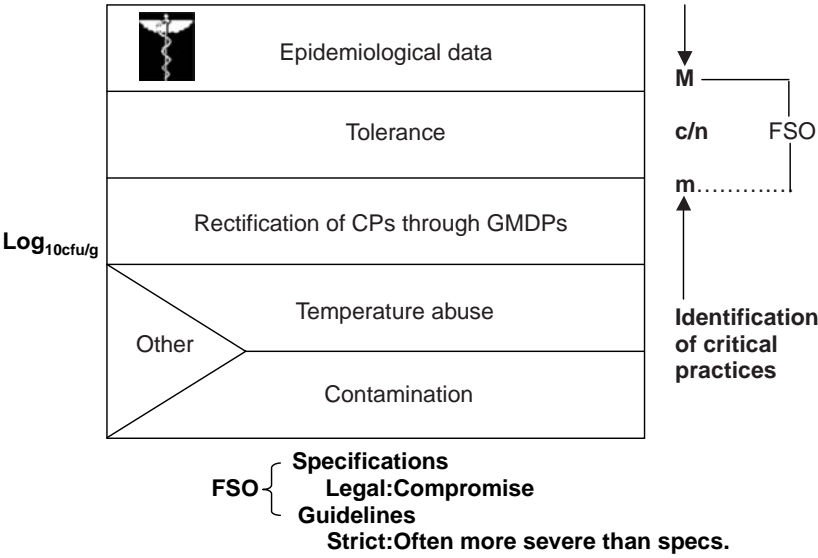


Figure 22.4. Risk analysis-based food safety objective (FSO) model proposed by Mossel et al. (1997). (Reproduced with permission from the Elsevier Publishers.)

the hazard to an acceptable low level. The goal in CCP determination is to use scientific management, and decision-making tools to determine critical processes which could be monitored and adequate control of such processes would reduce, minimize, or eliminate potential harm. A typical example as to how CCP determination is carried out is given in Table 22.21.

In the likely scenario of presence of *Staphylococcus aureus* (SA) or its toxin in frozen precooked chicken destined for further processing, the potential food safety hazard is not reasonably likely to occur because current contamination control, rapid CO₂ freezing, and good handling practices are adequate to control this hazard. Therefore, SA associated hazard does not need to be addressed as CCP. However, in case of frozen beef patties as well as egg containing food service preparation, *E. coli* and *Salmonella*-associated hazards are required to be addressed as CCP because severity of consequence and likelihood of hazard occurrence makes potential risk unacceptable (not instituting control of such grave risk is not tenable, hence this needs to be treated as CCP). Decision trees (Fig. 22.5) could be very useful in determining CCP. However, it should be kept in mind that they are merely tools and expert knowledge, experience, and rational, logical analysis are equally important in CCP determination exercise.

If prerequisite programs are not executed well or CCP determination is not sound, implementa-

tion of HACCP may not provide beneficial outcome. To emphasize criticality of sound CCP determination, NCIMS dairy HACCP requires the following verification

Section 3: HACCP Plan Critical Control Points

- ☐ A. HACCP plan lists CCP(s) for each food safety hazard identified as reasonably likely to occur.
- ☐ B. CCP(s) identified are adequate control measures for the food safety hazard(s) identified
- ☐ C. Control measures associated with CCP(s) listed are appropriate at the processing step identified

PRINCIPLE 3: ESTABLISH CRITICAL LIMITS

A critical limit is a maximum and or minimum value to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an ‘acceptable level’ the occurrence of a food safety hazard (FDA, 1997). At the heart of HACCP is verifiable monitoring regime that would help to control processing operation from unacceptable deviations. In other words, a proactive system analysis based on valid measures, limits of tolerance, and corrective measures is a key to institute self-check and timely interventions to preclude

Table 22.21. Critical Control Point Determination Generic Module Developed by National Advisory Committee on Microbiological criteria for Foods (NACMF)

Hazard Analysis Stage		Frozen Cooked Beef Patties Produced in a Manufacturing Plant	Product Containing Eggs Prepared for Foodservice	Commercial Frozen Precooked, Boned Chicken for Further Processing
Stage 1 Hazard identification	<i>Determine potential hazards associated with product</i>	Enteric pathogens (i.e., <i>Escherichia coli</i> O157:H7 and <i>Salmonella</i>)	<i>Salmonella</i> in finishe product.	<i>Staphylococcus aureus</i> in finishe product.
Stage 2 Hazard evaluation	<i>Assess severity of health consequences if potential hazard is not properly controlled.</i>	Epidemiological evidence indicates that these pathogens cause severe health effects, including death, among children and elderly. Undercooked beef patties have been linked to disease from these pathogens.	Salmonellosis is a foodborne infection causing a moderate to severe illness that can be caused by ingestion of only a few cells of <i>Salmonella</i> .	Certain strains of <i>S. aureus</i> produce an enterotoxin which can cause a moderate foodborne illness.
	<i>Determine likelihood of occurrence of potential hazard if not properly controlled.</i>	<i>E. coli</i> O157:H7 is of very low probability, and salmonellae are of moderate probability in raw meat.	Product is made with liquid eggs which have been associated with past outbreaks of salmonellosis. Recent problems with <i>Salmonella</i> serotype Enteritidis in eggs cause increased concern. Probability of <i>Salmonella</i> in raw eggs cannot be ruled out. If not effectively controlled, some consumers are likely to be exposed to <i>Salmonella</i> from this food.	Product may be contaminated with <i>S. aureus</i> due to human handling during boning of cooked chicken. Enterotoxin capable of causing illness will only occur as <i>S. aureus</i> multiplies to about 1,000,000/g. Operating procedures during boning and subsequent freezing prevent growth of <i>S. aureus</i> , thus the potential for enterotoxin formation is very low.
	<i>Using information above, determine whether this potential hazard is to be addressed in the HACCP plan.</i>	The HACCP team decides that enteric pathogens are hazards for this product.	HACCP team determines that if the potential hazard is not properly controlled, consumption of product is likely to result in an unacceptable health risk.	The HACCP team determines that the potential for enterotoxin formation is very low. However, it is still desirable to keep the initial number of <i>S. aureus</i> organisms low. Employee practices that minimize contamination, rapid carbon dioxide freezing and handling instructions have been adequate to control this potential hazard.
		Hazards must be addressed in the plan.	Hazard must be addressed in the plan.	Potential hazard does not need to be addressed in the plan.

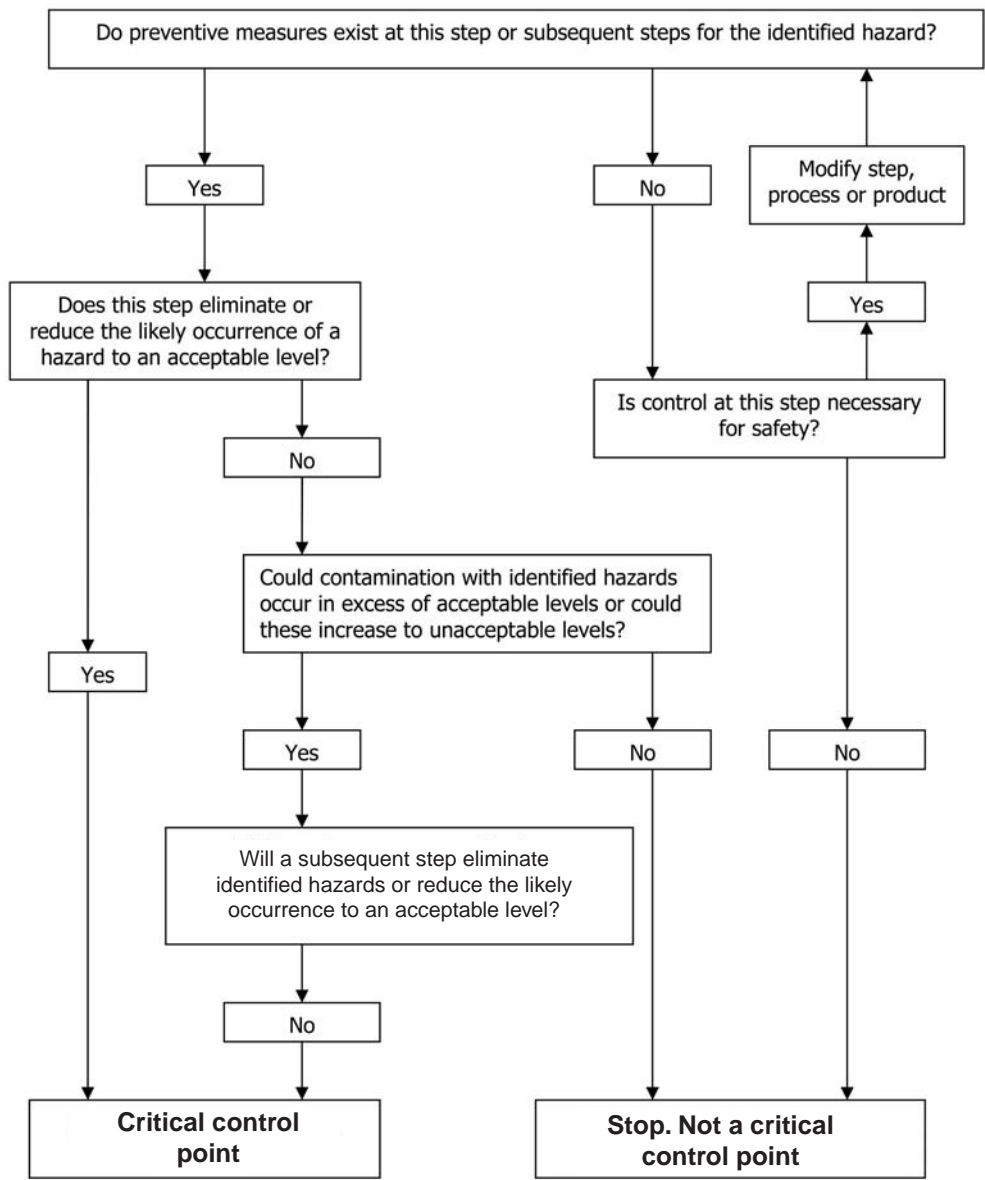


Figure 22.5. (a) CCP decision tree currently considered for dairy HACCP. (Source: FDA, 2003b, IDFA and NCIMS, 2006.)

harmful consequences. Also inherent in the system expectation is verifiability, auditing and validation measures that justify confidence in the model and its subtle outcomes. Hence, determination of rational, realistic, and science-based critical limits is a key to providing necessary teeth and arm to the HACCP program.

Each CCP might have one or more associated control measures. Each control measure would have one or more associated critical limits. In determining critical limits, regulatory standards or guidelines, scientific and technical literature, results of challenge studies, expert knowledge, and scientific risk analysis could serve as valuable resources. Setting critical

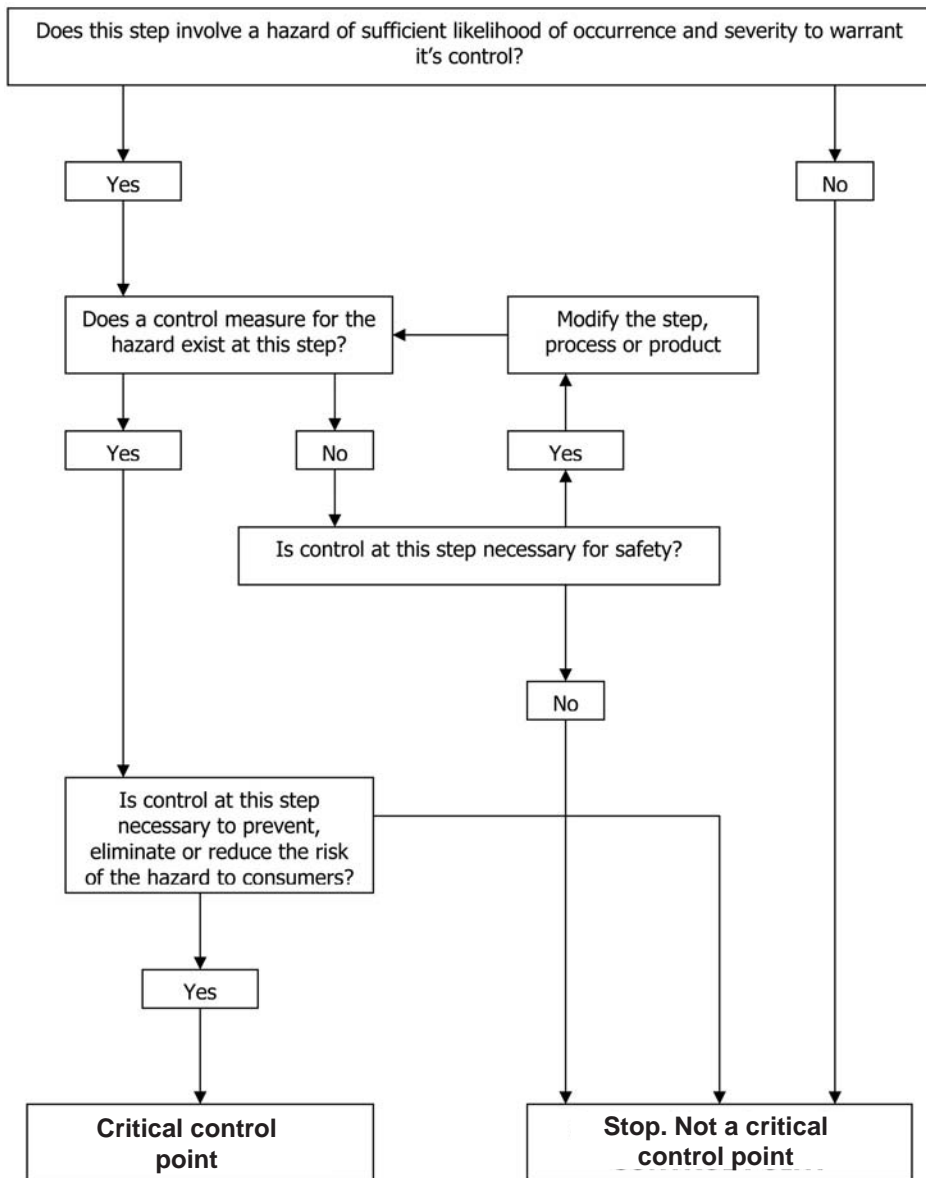


Figure 22.5. (b) Alternate CCP decision tree currently considered for dairy HACCP. (Source: FDA, 2003b, IDFA and NCIMS, 2006.)

limits is akin to benchmarking or establishing performance standards (Bernard and Scott, 2007). In a typical example of dairy processing, PMO specific pasteurization specification could serve as reference ranges, for example, temperature and time minima and phosphatase test, and so forth. Nevertheless, product ecology (pH, water activity, acidity,

salt/sugar concentration, humidity, moisture, viscosity, physical dimensions), thermobacteriology (thermal kill and resistance to kill), and sensory attributes are other important considerations in benchmarking critical limits. A 5-log reduction suggested by FDA for pathogen of concern in juice processing and a 12-D concept for botulinum safety in canned products

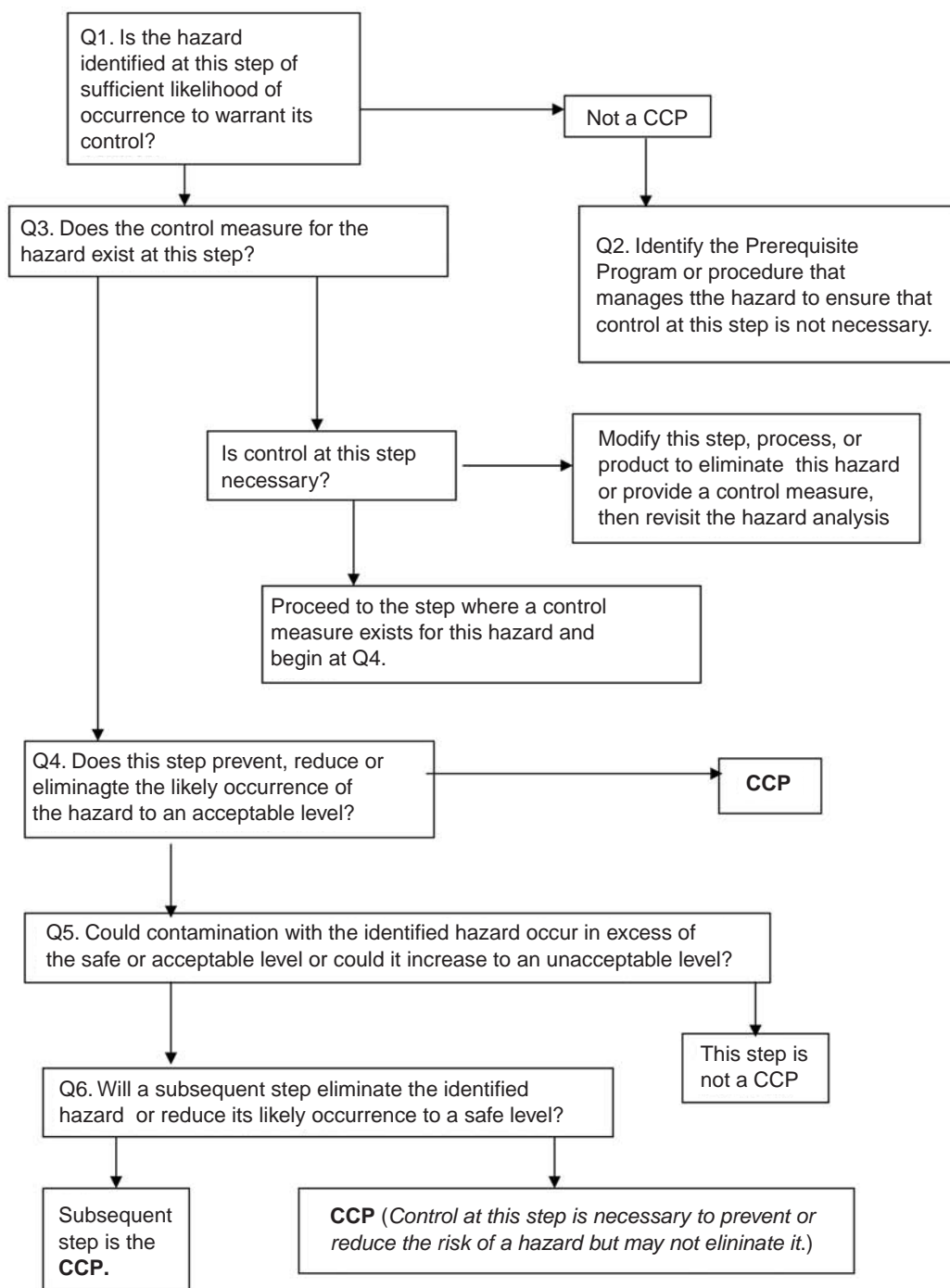


Figure 22.5. (c) Alternate CCP decision tree currently considered for dairy HACCP. (Source: FDA, 2003b, IDFA and NCIMS, 2006.)

Table 22.22. HACCP Critical Limit Determination Example

Critical Control Point (CCP)	Hazards	Critical Limits for Each Critical Control Point
Pasteurization (Properly functioning HTST without a magnetic flow meter system)	B—Vegetative pathogens	The temperature, as measured at the exit of the extended holding tube, must be at a minimum of 161°F.
Pasteurization (Properly functioning HTST with a magnetic flow meter system)	B—Vegetative pathogens	The temperature as measured at the exit of the extended holding tube, must be at a minimum of 161°F. The flow rate through the holding tube must meet the following criteria: Low Flow ___gpm High Flow ___gpm
Vat Pasteurization (with continuous agitation)	B—Vegetative pathogens	The temperature as measured by the air space indicating thermometer must be at a minimum of 145°F. The holding time must be a minimum of 30 minutes. The air space thermometer must indicate a minimum of 150°F.

Source: FDA (2007c,d,f).

could serve as satisfactory benchmarks. A typical example for fluid milk HACCP critical limit determination is shown in Table 22.22.

Time and temperature critical limits are set as minimum standards as per PMO guidelines.

To address essential need for setting efficacious critical limits in dairy HACCP program, NCIMS requires conformance as to whether:

- ☐ A. HACCP plan lists critical limits for each CCP.
- ☐ B. CL(s) are adequate to control the hazard identified
- ☐ C. CL(s) are achievable with existing monitoring instruments or procedures.
- ☐ D. CL(s) are met.

Presently, microbiological criteria as critical limits is not preferable due to longer time required for analysis coupled with relative lack of sensitivity and specificity (affecting reliability and hence, decision making). However, with advances in rapid methods microbiology and emerging biosensor-nanotechnology platforms, the situation about microbiological criteria as critical limits is likely to change.

PRINCIPLE 4: ESTABLISH MONITORING PROCEDURES

Monitoring is a planned sequence of observations or measurements to assess whether a CCP is under control or it is deviating beyond preset tolerance levels. Data generated during monitoring are also useful for other essential functions of HACCP such as

verification and validation. Commonly used monitoring procedures include temperature (temperature recording thermograph or thermometer reading) and time measurement, pH measurement, water activity measurement, and so forth.

One of the salient features of HACCP is its intelligible, system management logic. Inherent in the HACCP ideology is:

1. Continuous process assessment. Constantly asking as to whether processing operation is performing as normally as designed.
2. Continuous assessment of reliability, precision, fitness and integrity of system components in providing high confidence objective output.

For monitoring procedures to be effective, their proper assignment and calibration, recording and data retrieval training, are important. Where possible, it is best to have continuous monitoring of CCP step as it reflects on-line, “real time” manifest. However, it may not be always feasible to have continuous monitoring of certain parameters viz. pH, water activity measurement. In such situations, monitoring frequency should be rational and logical to justify benefit of repeated monitoring. Generic CCP monitoring frequency encountered in fluid milk processing operation and cheese processing are shown in Tables 22.23 and 22.24.

Organizing each CCP monitoring in specific details (who, what, how, and when) is preferable to avoid oversight or chance error.

Table 22.23. Typical Monitoring Procedures in a Cheese Operation

CCP Step	Potential Hazard	Control Criteria	Critical Limit	Monitor Frequency	Records (Location)	Responsibility	Corrective Action	Verification
Raw milk receiving	Biological	Temp.	<45°F	Every tanker	Load ticket (QA/QC)	Intake operator	Hold and evaluate	Thermometer
	Drug residues	Antibiotic screening	No positives	Every tanker	Receiving log QA/QC	Intake operator	Reject milk	Calibrate kit
Dairy ingredient storage	Biological	Temp. Time	<45°F <72 hours	3 times Daily	Recording chart	QA Tech.	Hold, investigate	Recording indicating Thermometer
Raw Cream Storage	Biological	Temp. Time	<45°F <72 hours	3 times Daily	Recording chart QA/QC	QA Tech.	Hold, investigate	Recording indicating Thermometer
Pasteurization	Biological	Temp. Time	≥161°F ≥15 seconds	Continuous	Recording chart. Production office	Pasteurizer operator	Flow divert, recirculate and heat	Cut-in/cut-out checks, thermometer calibration
Culture media pasteurization	Biological	Temp. Time	≥161°F ≥15 seconds	Continuous	Recording chart. Production office	Pasteurizer operator	Flow divert, recirculate and heat	Cut-in/cut-out checks, thermometer calibration
Milling	Biological	Acid development	pH <5.60 after culture	Every vat	Recording chart QA/QC	QA Tech	Test for <i>S. aureus</i> if pH >5.60	Calibrate pH meter each time
Metal detector	Physical	Metal fragments	Limit of detection	Continuous	Detector Log QA/QC	Filling operator	Reject. Locate cause	Detector calibration

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Table 22.24. Monitoring Procedures for a Dairy Processing Operation

Monitoring				
Critical Control Point (CCP)	WHAT	HOW	FREQUENCY	WHO
Pasteurization (Properly functioning HTST without a magnetic fl w meter system)	Temperature (°F)	Check and sign-off on temperature recording charts.	Monitoring is done by operator every 2 hours and after each product run.	Pasteurizer/Operator
Pasteurization (Properly functioning HTST with a magnetic fl w meter system)	Temperature (°F) Flow Rate between low fl w set point and high fl w set point (gpm)	Check and sign-off on temperature and fl w recording charts.	Monitoring is done by the operator after every 2 hours and after each product run.	Pasteurizer/Operator
Vat Pasteurization (with continuous agitation)	Temperature (°F) Time (min) Time (min)	Check and sign-off on recording charts. Record both air space and indicating thermometer temperatures.	Thermometer checks done at beginning and end of holding time. Annotate the batch information for each batch on the recording chart.	Pasteurizer/Operator

Source: FDA, 2007c,d,f.

Where possible, assigning monitoring responsibility to floor personnel and delegating authority to shut down production line could serve as positive motivating factors. NCIMS dairy HACCP aims to verify following important aspects of monitoring regime employed by a processing facility.

Section 5: HACCP Plan Monitoring

- ☐ A. HACCP plan define monitoring procedures for each CCP (what, how, frequency, whom).
- ☐ B. Monitoring procedures as define in the HACCP plan followed.
- ☐ C. Monitoring procedures as define in the HACCP plan adequately measure critical limits at each CCP.
- ☐ D. Monitoring record data consistent with the actual value(s) observed during the audit.

PRINCIPLE 5: ESTABLISH CORRECTIVE ACTIONS

As we have seen by now, HACCP is a predominantly proactive and preventive system. Logistics and technical resources are employed to effectively track CCPs as per established monitoring procedures. As a consequence of monitoring, following three outcomes are possible:

1. Process worked within expected tolerance range.
2. Slight deviations were noticed at some time points before correction was made.
3. Large deviations were noticed before correction was made.

Situations 2 and 3 above would warrant professional decision based on science, rationality, and transparent logic. Subjective variations in the decision making would compromise consistency and performance in attaining food safety objective. It is imperative then to develop a research-based policy addressing normal variations as opposed to unacceptable variations. It should be clearly laid out as to how to treat unsatisfactory product. Such an exercise of formulating timely and clear corrective actions is what constitutes HACCP essential Principle 5. Clear policy and written procedures for corrective actions is instrumental in deriving consistent and accurate food safety decisions, avoiding costly blunders. Having a written record of corrective actions and procedures is emphasized in NCIMS dairy HACCP by requiring that:

- ☐ A. Corrective actions when define in the HACCP plan were followed when deviations occurred.
- ☐ B. Predetermined corrective actions define in the HACCP plan ensure the cause of the deviation is corrected.
- ☐ C. Corrective action taken for products produced during a deviation from critical limits define in the HACCP plan.
- ☐ D. Affected product produced during the deviation segregated and held, AND a review to determine product acceptability performed, AND corrective action taken to ensure that no adulterated and/or product that is injurious to health enters commerce.
- ☐ E. Cause of deviation was corrected.
- ☐ F. Reassessment of HACCP plan performed and modified accordingly.
- ☐ G. Corrective actions documented.

Having a clear record about deviations and corrective actions in form of a centralized deviation log, as shown in a following generic example, is a practical proof of functional HACCP (see Table 22.25).

One of the salient aspects of HACCP system is that it is an evolving system and there is a continuous learning curve involved at all levels of its implementation. Sophistication of HACCP implementation by a processing facility is reflected in how deviations are addressed and adequately documented for further research and review.

PRINCIPLE 6: ESTABLISH VERIFICATION PROCEDURES

Verification is defined as those activities, other than monitoring, that aim to determine whether HACCP system is designed adequately, working as designed and whether there are any flaws that are discernible. Verification is also described as secondary review as opposed to primary review (monitoring) conducted by line personnel (Bernard and Scott, 2007). Typically, the responsibility for secondary review is that of quality assurance to verify that records are being kept accurately and that monitoring is done satisfactorily.

As a science-based and management system, HACCP aims to incorporate systematic, technical, and administrative measures to build integrity, precision, reliability, and validity in its performance. To address this, typical verification regime includes in-plant observations, measurements, evaluations, expert advice, and scientific studies.

Table 22.25. Critical Limit Deviation and Corrective Action Log

Deviation #1	
TODAY’S DATE:	DATE OF INCIDENT:
DATE REPORTED:	REPORTED BY:
EXPLAIN CCP CRITICAL LIMIT DEVIATION:	
PRODUCT/PROCESS INVOLVED	
<ul style="list-style-type: none">• Product Name and Description:• Code Date(s):• Date(s) of Manufacture:• Production Line #:	
CORRECTIVE ACTION:	ACTION TAKEN
1. Segregate and hold the affected product until 2. and 3. are completed	<input type="checkbox"/> Yes <input type="checkbox"/> No Date: Comments
2. Perform or obtain a review to determine the acceptability of the affected product for distribution. The review shall be performed by an individual or individuals qualified by training or experience to perform such a review;	<input type="checkbox"/> Yes <input type="checkbox"/> No Date: Comments
3. Take corrective action, when necessary, with respect to the affected product to insure that no product is allowed to enter commerce that is either injurious to health or is otherwise adulterated as a result of the deviation;	<input type="checkbox"/> Yes <input type="checkbox"/> No Date: Comments
4. Take corrective action, when necessary, to correct the cause of the deviation; and	<input type="checkbox"/> Yes <input type="checkbox"/> No Date: Comments
Perform or obtain timely validation by a qualified individual(s), as required in Appendix K, to determine whether modification of the HACCP Plan is required to reduce the risk of recurrence of the deviation, and modify the HACCP Plan as necessary.	<input type="checkbox"/> Yes <input type="checkbox"/> No Date: Comments
DISPOSITION OF PRODUCT	ROOT CAUSE OF DEVIATION

Source: FDA, 2007a,g.

Microbiological testing as a monitoring tool may not be practicable; however, microbiological criteria as a verification/ validation tool may be quite appropriate. Though end-product testing is expected to be minimal, intelligently designed testing regimens with high tech power of rapidity, reliability, and efficiency of modern methods could provide useful validation and verification data for the HACCP system. Trend analysis and other statistical data analysis tools could also provide additional confidence and predictable avenues for timely actions before having to face costly recalls/lawsuits. If power of nanotechnology and biosensor research is any indication, sensor-based microbial food process monitoring

and verification system should greatly impact HACCP. A system based on real-time monitoring and microbial-based validation is expected to reduce food safety failure rate. The take-home message here is: Based on current research in predictive microbiology, microbial risk modeling, and advances in rapid and automated detection technologies, it is to be expected that future HACCP validation and verification steps would involve powerful tools of modern microbiology.

A typical HACCP verification schedule followed in dairy processing is given in Table 22.26.

NCIMS review plan takes into account any product/process changes, CCP deviations, recalls,

Table 22.26. Typical HACCP Verification Schedule

Activity	Frequency	Responsibility	Reviewer
Verification activities scheduling	Yearly or upon HACCP system change	HACCP coordinator	Plant manager
Initial validation of HACCP plan	Prior to and during initial implementation of plan	Independent expert(s) ^a	HACCP team
Subsequent validation of HACCP plan	When critical limits changed, significant changes in process, equipment changed, after system failure, etc.	Independent expert(s) ^a	HACCP team
Verification of CCP monitoring as described in the plan (e.g., monitoring of patty cooking temperature)	According to HACCP plan (e.g., once per shift)	According to HACCP plan (e.g., line supervisor)	According to HACCP plan (e.g., quality control)
Review of monitoring, corrective action records to show compliance with the plan	Monthly	Quality assurance	HACCP team
Comprehensive HACCP system verification	Yearly	Independent expert(s) ^a	Plant manager

^a Done by others than the team writing and implementing the plan. May require additional technical expertise as well as laboratory and plant test studies.
Source: FDA, 1997.

regulatory, advisories, food safety-related consumer concerns and systematic review about entire HACCP plan including adequacy of existing CCP, monitoring, and verification measures (see Table 22.27).

PRINCIPLE 7: ESTABLISH RECORD KEEPING AND DOCUMENTATION PROCEDURES

Documentation and efficient record keeping has its own significance in a safety management system. As a systematic tool to enhance food safety, technological and instrumental resources are integrated to meet business needs. Alongside technological and safety management apparatus, human element is intimately involved. Appropriate actions, professional decisions, and hands-on food safety practices heavily rely on human interface, and human beings have obvious limitations. Proper documentation and systematic records help to reduce human errors to minimum. One of the distinct features of operational parameter of HACCP is its checklist, self-check type methodical approach, comparable to *pill organizer* or *pill*

reminder system. Practical experience amply testify usefulness of such simple but sensible methodical approach. Over and above minimizing human errors in HACCP management, record keeping, and documentation accomplishes following important functions:

1. Facilitates continuous review and verification
2. Creates knowledge resource for critical evaluations and continuous improvements.
3. Fulfill regulatory requirements.
4. Helps investigation in case of specific problems.
5. Serves as documentary evidence for responsible care.
6. Strengthens objective and rational evaluation (facts and figure speak themselves!) enhancing individual cum collective responsibility.
7. A transparent tool open to criticism, review, and rightful professional pride.

NCIMS dairy HACCP recommends extensive list of documents and records as outlined in Table 22.28. Documentation and records pertaining to prerequisite programs and essential HACCP documents make food safety review easy, practicable, and efficacious

Table 22.27. Typical Verification and Validation Check List Proposed Under NCIMS

Topic	Yes	No	If “Yes,” Describe	Food Safety Implication?	Are modification to the HACCP system required?
1. Evaluate product and process					
Product description changed, e.g., intended use, consumer?	<input type="checkbox"/>	<input type="checkbox"/>			
Formula changed?	<input type="checkbox"/>	<input type="checkbox"/>			
Ingredients/Packaging changed?	<input type="checkbox"/>	<input type="checkbox"/>			
Any new product consumption or storage methods?	<input type="checkbox"/>	<input type="checkbox"/>			
Any new suppliers?	<input type="checkbox"/>	<input type="checkbox"/>			
Process fl w changed?	<input type="checkbox"/>	<input type="checkbox"/>			
Equipment/Computer software changed?	<input type="checkbox"/>	<input type="checkbox"/>			
Finished product distribution changed?	<input type="checkbox"/>	<input type="checkbox"/>			
Other, e.g., production volume increased:	<input type="checkbox"/>	<input type="checkbox"/>			
2. Evaluate product/process history					
Repeat CCP deviations?	<input type="checkbox"/>	<input type="checkbox"/>			
Any recent industry recalls of similar product since the last annual validation?	<input type="checkbox"/>	<input type="checkbox"/>			
New or emerging hazards, e.g., recent CDC Morbidity and mortality problems identifie with product?	<input type="checkbox"/>	<input type="checkbox"/>			
Regulatory agency recommendations, e.g., guidance documents, regulations?	<input type="checkbox"/>	<input type="checkbox"/>			
Any confirme food safety consumer complaints?	<input type="checkbox"/>	<input type="checkbox"/>			
3. Evaluate adequacy of CCPs, critical limits, monitoring, corrective action, CCP verification and record keeping procedures. Review current CCP documentation.					
Do the CCPs control the hazards?	<input type="checkbox"/>	<input type="checkbox"/>			
Are the CCP critical limits adequate?	<input type="checkbox"/>	<input type="checkbox"/>			
Do monitoring methods and frequency demonstrate control?	<input type="checkbox"/>	<input type="checkbox"/>			
Do corrective actions properly address affected product and correct deviations?	<input type="checkbox"/>	<input type="checkbox"/>			
Does validation include review of consumer complaints?	<input type="checkbox"/>	<input type="checkbox"/>			
Other, e.g., Prerequisite Programs or procedures may affect the hazard analysis	<input type="checkbox"/>	<input type="checkbox"/>			

Source: FDA, 2007h.

Table 22.28. Centralized List of HACCP Records

SUBJECT	ISSUE DATE		
Centralized List of HACCP Program Records PLANT NAME ADDRESS	SUPERSEDES	PAGE xvii of 1	
The purpose of this checklist is to assist the plant HACCP team in demonstrating that those records normally required under the NCIMS HACCP voluntary alternative are current and available. This checklist may also serve as a tool for internal and external HACCP auditors.			
Record	Available (√ = yes)	Most Current Version (√ = yes)	Comments
Required HACCP documents including forms are dated or identified with current version number. Each page is marked with a new date or version number whenever that page is updated. Most current versions used.		NA	
Table of Contents			
Centralized List of HACCP Program Records			
Document Change Log			
Process Flow Diagram(s)			
Product Description(s)			
Written Hazard Analysis(s) for each product			
CCP HACCP Plan Summary(s) for each product			
CCP Monitoring Documents			
Centralized Deviation Log			
HACCP System Verification Documentation (including calibration of CCP monitoring equipment (i.e., past equipment checks); review of CCP monitoring records, corrective action records, and calibration records; and plant signatures and date on these records)			
HACCP System Validation Documentation (Annually or when changes are made in raw materials or source of raw materials; product formulation; processing methods or systems, including computers and their software; packaging; finished product distribution systems; or the intended use or intended consumers of the finished product and consumer complaints)			

Table 22.28. (cont.)

Record	Available (✓ = yes)	Most Current Version (✓ = yes)	Comments
Prerequisite Program #1 – Safety of Water			
•Monitoring Records related to this PP (list records by name)			
•Nonconformity correction records related to this PP			
Prerequisite Program #2 – Condition and Cleanliness of Food Contact Surfaces			
•Monitoring Records related to this PP			
•Nonconformity correction records related to this PP			
Prerequisite Program #3 – Prevention of Cross-Contamination			
•Monitoring Records related to this PP			
•Nonconformity correction records related to this PP			
Prerequisite Program #4 – Maintenance of Hand Washing and Sanitizing and Toilet Facilities			
•Monitoring Records related to this PP			
•Nonconformity correction records related to this PP			
Prerequisite Program #5 – Protection from Adulteration			
•Monitoring Records related to this PP			
•Nonconformity correction records related to this PP			
Prerequisite Program #6 – Proper Labeling, Storage, and Use of Toxic Compounds			
•Monitoring Records related to this PP			
•Nonconformity correction records related to this PP			
Prerequisite Program #7 – Control of Employee Health Condition			
•Monitoring Records related to this PP			
•Nonconformity correction records related to this PP			

Table 22.28. (cont.)

Record	Available (✓ = yes)	Most Current Version (✓ = yes)	Comments
Prerequisite Program #8 – Exclusion of Pests			
• Monitoring Records related to this PP			
• Nonconformity correction records related to this PP			
Other Prerequisite Programs that are relied upon in the Hazard Analysis to reduce the likelihood of a potential hazard (List each separately, add rows as needed)			
• Monitoring Records related to this PP			
• Nonconformity correction records related to this PP			
Other Applicable NCIMS Requirements – Appendix K (list each separately, add rows as needed)			
• Monitoring Records related to these requirements			
• Nonconformity correction records related to these requirements			

Source: FDA, 2007e.

DEMING'S QUALITY DOCTRINE

According to Merriam Webster dictionary, quality is defined as a character or attribute perceived to be superior, essential, and distinct. Quality is best described as a perception turned to reality in the minds of consumer. It is difficult to measure or describe quality since it is a subjective term. Nevertheless, quality is a defining face of modern business. Consumer appeal of business services and growth/survival of business is dependent upon delivering quality. However, concept of quality was not well defined during early part of industrial revolution and mass production. Development of quality concept is attributed to early statisticians namely Douglas McArthur, Walter Shewhart, and William Edward Deming who applied statistical process control methods such as ANOVA, hypothesis testing, and so forth, to improve product quality (Deming, 1975).

Dr. Deming's work on quality as a process control as well as modern management tool is so profound that he is considered father of quality (Fig. 22.6).

Dr. Deming lectured extensively and his famous quotes, which might be relevant even today for the food processing industry, are summarized in Table 22.29.

Dr. Deming's work was most appreciated in Japan. Concepts of Kaizen, and Six Sigma were direct outcomes of Dr. Deming's teaching of statistical process control principles to Japanese industry (Fig. 22.7).

A number of Japanese companies experienced surge in quality and productivity as a direct result of Deming's quality doctrine. The improved quality combined with lower costs created huge international demand for Japanese products. For his outstanding contribution to Japanese industrial growth and robust economy, he was conferred Japan's Order of Sacred Treasures Civilian award. The Japanese industry has instituted Deming Prize, which is given out to companies that exhibit significant influence on quality control and quality management in Japan.

It is becoming apparent that modern quality management principles are intimately associated with quality philosophy of Dr. Deming as could be seen in his concept of *profound knowledge* (Table 22.30.).



Figure 22.6. William Edward Deming (1900–1993) is considered *Father of Modern Quality*. Dr. Deming traveled and lectured extensively and influenced management and process control methodologies. (Photo Courtesy Diana Deming Cahill, Deming Institute, Potomac, MD.)

Quality movement developed as a sound statistical process control methodology. Modern food safety movement capitalized on several tools available in quality methodology such as root cause analysis, FMEA, Six Sigma, Pareto chart, trend analysis, and risk analysis, and so forth. However easy it might seem, integration of food safety principles and quality management tools is one of the most practical and trend-setting accomplishments of HACCP pioneers.

KAIZEN, SIX SIGMA, AND ITS RELEVANCE TO FOOD SAFETY AND QUALITY

Kaizen and Six Sigma concepts developed as management principles influence by Deming's statistical quality control methodologies. Ground breaking as it was, Deming's application of quality principles to not only production situations but also, business functions, lead to management revolution of its kind. Kaizen ideology focuses on employee component for process improvements whereas Six Sigma emphasizes robust process control by sound statistics. When applied together, both tend to optimize personnel-process dynamics to improve end-results. According to Department of Japan Human Resources, Kaizen and Six Sigma are not cultural

concepts but robust management principles. Hence, there is no reason why such successful models could not be employed elsewhere!

Kaizen literally means *continuous improvement or change-for-the-better*. Kaizen philosophy calls for *never-ending efforts* for improvement by one and all, managers as well as workers. At the heart of Kaizen is quality, productivity, and employee participation (through motivation and work satisfaction). The beauty of Kaizen concept lies in its simple logic of small but continuous improvements that ultimately lead to superior results and productivity as a compound effect. This is akin to Mr. X aiming to call 100 persons would not be able to do the job for months but could do so easily in a month were he to call 3–4 contacts everyday on a consistent basis. Optimum size and consistency tends to develop its own critical mass. This seemingly simple concept has revolutionized Japanese company's performance and achievement charts. Kaizen ideology and its successful integration in management is illustrated in Figures 22.8 and 22.9.

Employee participation and motivation is a huge part of Kaizen. Kaizen ideology preaches that employee participation is not influence by money as much by psychological factors such as ability to contribute and freedom to innovate and experiment (albeit within realms of practicality and within the context of productivity/quality). This concept is not alien to the U.S. management as already observed in Hawthorne principle, wherein employees given adequate attention (motivation) produced better output irrespective of actual level of comfort (adequate lighting arrangement). In the context of food processing and team setups, employee motivation and participation become important factors. For example, sanitation, monitoring, verification and inspection activities associated with quality control/HACCP are heavily impacted by wholehearted involvement of all the crew at all levels. Thus, professional pride and diligent work performance for the assigned task should be encouraged and promoted.

In contrast to Kaizen, Six Sigma aims to improve process performance by minimizing process variations from specification. The basic premises of Six Sigma are:

- Manufacturing and business processes are measurable and analyzable and hence they could be controlled and improved.
- Continuous efforts to reduce variation in process is a key to success.

Table 22.29. Comparison of Deming’s Quality Doctrine with Modern Food Safety and Quality Management Structure

Famous Quote	Current Food Safety Context or Relevance
There is no substitute for knowledge	Continuous training, learning opportunities. Continuous improvements and knowledge organizations
The most important things cannot be measured. The most important things are unknown or unknowable	Quality and food safety are not absolute. They are rather abstract concepts. Definining and refining quality and/or safety parameters is an ongoing quest.
Experience by itself teaches nothing	One needs to interpret and apply information against a theory or framework of concepts that is the basis for knowledge about a system
You can expect what you inspect	You cannot inspect quality. You have to build one in.
System => input + process = output, all 3 are inspected to some degree. By inspecting the inputs and the process more, the outputs can be better predicted and inspected less. Rather than inspection of all the product, the output can be statistically sampled in a cause–effect relationship through the process	HACCP is a proactive system aimed at preventive management. End product testing is minimal through better process control verification and validation measures
Acceptable defects. Rather than waste efforts on zero-defect goals, one must stress the importance of establishing a level of variation, or anomalies, acceptable to the customer in the next phase of a process	Setting realistic food safety objective to meet food safety goals for a population exposed to a product over given time
The problem is at the top; management is the problem	For HACCP or quality control to be successful, management commitment is necessary
A system must be managed. It will not manage itself	One must get above paper quality. Functional and working HACCP system needs efforts and desire to raise the bar.
What is the variation trying to tell us about a process, about the people in the process?	Chance causes = common causes = management domain. Assignable causes = process, worker, middle management and supervisor domain

Source: Deming Institute and Vadim Kotelnikov (Personal communication).

Six Sigma concept is developed by Motorola Inc. According to Motorola University dictionary, the term *Six Sigma* refers to the ability of highly capable processes to produce output within specification. In particular, processes that operate with Six Sigma quality produce defect levels below 3.4 defects per million opportunities. Six Sigma is based on basic methodology developed by Dr. Deming, that is, Plan-Do-Check-Act Cycle. Other tools used for Six Sigma include failure mode and effects analysis, histograms, regression analysis, analysis of variance, cause and effects analysis, chi-square test of independence and fits correlation and cost–benefit analysis, and so

forth. As discussed previously, statistical tools described above played critical role in developing risk analysis thinking central to HACCP model. With developments in detection technology platforms and real-time analysis of data capability, statistical process control would be expected to play important role in food safety management.

CODEX STANDARDS AND THE GLOBAL FOOD TRADE

Codex food standards originally started as a European framework of food laws to facilitate



Figure 22.7. Dr. Deming during his trip to Japan giving his presentation to management. (Photo Courtesy Diana Deming Cahill, Deming Institute, Potomac, MD.)

intercountry trade. However, with international consensus, initiatives and fruitful deliberations; Codex standards are now a framework of regulations governing international food trade (Table 22.31).

It is indeed a remarkable achievement of the twenty-first century under the auspices of FAO and WHO of the United Nations that scientific, technical, and political leadership have come together to enact such comprehensive yet flexible food standards. Codex Alimentarius Commission is charged with administering the food regulations under the joint authority of WHO, FAO, and ISO. One of the unique features of Codex standards is that it is a sort of one-stop solution to food regulations wherein quality and food safety standards and specification are laid out with explicit rules for harmonization, dispute settlement, and removing

Table 22.30. Deming's 14 Point of *Profound Knowledge* (Also Called TQM)

1. Create constancy of purpose toward improvement of a product and service with a plan to become competitive and stay in business. Decide to whom top management is responsible (Consumers. . . Stakeholders!!).
2. Adopt the new philosophy. We are in a new economic age. We can no longer live with commonly accepted levels of delays, mistakes, defective materials, and defective workmanship.
3. Cease dependence on mass inspection. Require instead, statistical evidence that quality is built in (prevent defects instead of detect defects).
4. End of the practice of awarding business on the basis of price tag. Instead, depend on meaningful measures of quality along with price. Eliminate suppliers that cannot qualify with statistical evidence of quality.
5. Find Problems. It is a management's job to work continually on the system (design, incoming materials, composition of material, maintenance, improvement of machine, training, supervision, retraining).
6. Institute modern methods of training on the job.
7. The responsibility of the foreman must be to change from sheer numbers to quality. . . [which] will automatically improve productivity. Management must prepare to take immediate action on reports from the foremen concerning barriers such as inherent defects, machines not maintained, poor tools, and fuzzy operational definitions
8. Drive out fear, so that everyone may work effectively for the company.
9. Break down barriers between departments. People in research, design, sales and production must work as a team to foresee problems of production that may be encountered with various materials and specifications
10. Eliminate numerical goals, posters, slogans for the workforce, asking for new levels of productivity without providing methods.
11. Eliminate work standards that prescribe numerical quotas.
12. Remove barriers that stand between the hourly worker and his right of pride of workmanship.
13. Institute a vigorous program of education and retraining.
14. Create a structure in top management that will push every day on the above 13 points.

Source: Deming Institute and Vadim Kotelnikov (Personal communication).

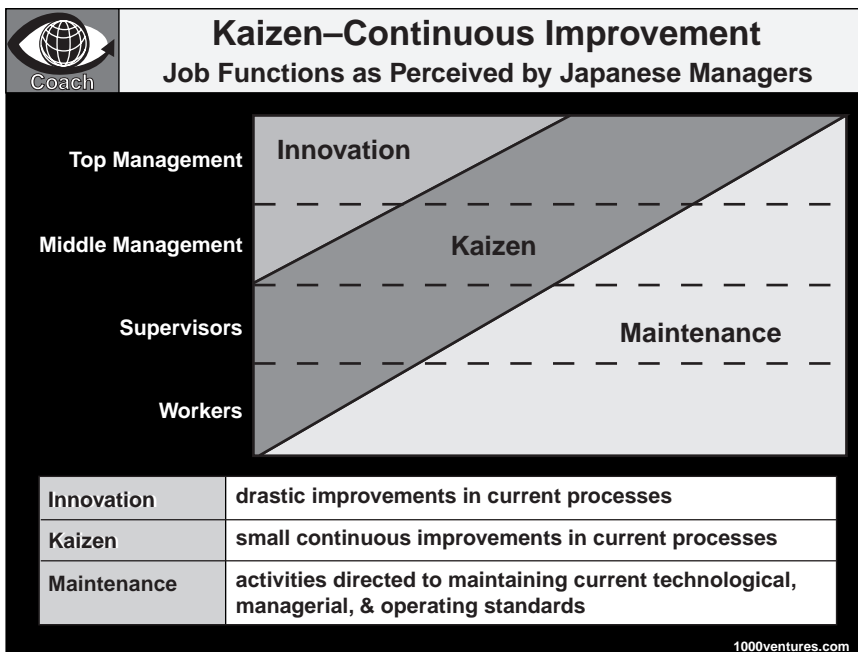


Figure 22.8. Relevance of Kaizen for middle management, supervisors and workers.

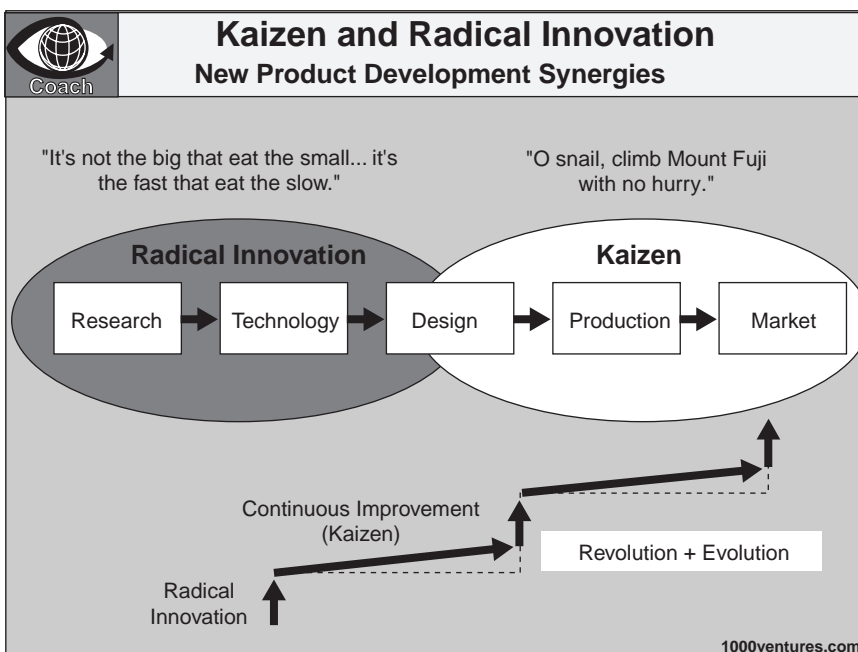


Figure 22.9. Integration of R&D and product performance for delivering quality.

Table 22.31. Codex Alimentarius Commission Milestones

1800s	General food laws and enforcement agencies.
Early 1900s	Food trade associations involved in harmonized food standards.
1903	The International Dairy Federation (IDF) developed global standards for milk and milk products.
1943	United States President Franklin D. Roosevelt convened FAO's founding conference, the United Nations' Conference on Food and Agriculture, in Hot Springs, Virginia.
1945	FAO is founded. Its responsibility included nutrition and international food standards.
1948	WHO is founded. Its responsibility included human health and food safety standards.
1950	Joint FAO/WHO expert meetings begin on nutrition, food additives, and related areas.
1954–1958	Austrian initiative for a regional food code, the <i>Codex Alimentarius Europaeus</i> , or European Codex Alimentarius.
1960	FAO Regional Conference for Europe endorsed need for an international food code.
1961	Director General of FAO, B.R. Sen, actively pursued discussions with WHO and the Codex Alimentarius Europaeus that would ultimately lead to the establishment of an international food standards program. <i>Codex Alimentarius Europaeus</i> hands over food standards work to FAO/WHO.
1962	The Joint FAO/WHO Food Standards Conference created Codex Alimentarius with a mandate to implement FAO/WHO joint food standards.
1985	UN general assembly guidelines emphasized need of all consumers for food security while formulating national policies and plans with regard to food.
1991	The FAO/WHO Conference on Food Standards (in cooperation with GATT) agreed to accelerate the process of harmonizing national food regulations to bring them into line with international standards and with particular focus on consumer protection (health and food safety).
1992	The FAO/WHO International Conference on Nutrition declared that access to nutritionally adequate and safe food is a right of each individual.
1995	The agreement on sanitary and phytosanitary measures and technical barriers to trade formalized international standards, guidelines and recommendations by Codex Alimentarius as reference points for facilitating international trade and resolving trade disputes in international law.
1995	Consultation on Risk Assessment and Food Safety.
1996	Consultation on Biotechnology and Food Safety.
1997	Consultation on the Application of Risk Management to Food Safety.
1998	Consultation on the Role of Government Agencies in Assessing HACCP. Consultation on the application of risk communication to food standards and safety matters.

Source: FAO (1995, 1999), miscellaneous sources.

restrictive and unfair barriers to free trade on the basis of sound science and coherent logic. In fact, Codex standards have become the benchmarks against which national food measures and regulations are evaluated within the legal parameters of the international trade agreements.

MAIN FUNCTIONS OF CODEX COMMISSION

Codex Alimentarius Commission has drawn global attention to the field of food quality and safety. During the past three decades, food regulations pertaining to

the protection of consumer health and fair practices in the food trade have been handled by Codex. The agency has fostered scientific and technological research as well as discussion. In doing so, it has lifted the world community's awareness of food safety and related issues to remarkable heights. Not surprisingly, then Codex has become the single most important international reference point for international food trade and associated standards.

Over and above developing and implementing food standards and specifications Codex commission is involved in the following academic/research activities:

- Convening expert meetings, for example, Joint FAO/WHO Meeting on pesticide residues.
- Conducting workshops and training courses for transferring information, knowledge, and skills as well as to promote awareness of the commission.
- Improving laboratory analysis and food inspection procedures.
- Presenting papers at conferences, meetings, and symposia on the relevance of Codex activities to the provision of safe food of acceptable quality.
- Extending guidance on matters directly related to Codex activities, for example, safety assessment of food produced using biotechnology.
- Developing and publishing manuals and texts that are associated with food quality control and safety systems.
- Developing and publishing training manuals on food inspection and quality and safety assurance, particularly with respect to the HACCP system in the food-processing industry (FAO, 1995, 1999).

FUNCTIONAL STRUCTURE OF CODEX

Codex Alimentarius Commission secretary is a senior FAO official who serves as the Chief of the Joint FAO/WHO Food Standards Program, located within the Food Quality and Standards Service of the Food and Nutrition Division at FAO in Rome. The Codex function is derived from its mandate of statutes, rule of procedure, and representational participation. Development and revision of standards is an open and transparent procedure that involves proposal submission followed by decision by commission and draft standard circulation for comments. Standards and

regulations are revised and updated to maintain relevance and adequacy of such measures.

Working structure of the Codex Alimentarius:

- Volume 1A—General requirements
 - General Principles of the Codex Alimentarius
 - Definition for the Purpose of Codex Alimentarius
 - Code of Ethics for International Trade in Foods
 - Food Labeling
 - Food Additives—including the General Standard for Food Additives
 - Contaminants in Food—including the General Standard for Contaminants and Toxins in Foods
 - Irradiated Foods
 - Food Import and Export Food Inspection and Certification Systems
- Volume 1B—General requirements (food hygiene)
- Volume 2A—Pesticide residues in foods (general texts)
- Volume 2B—Pesticide residues in foods (maximum residue limits)
- Volume 3—Residues of veterinary drugs in foods
- Volume 4—Foods for special dietary uses (including foods for infants and children)
- Volume 5A—Processed and quick-frozen fruits and vegetables
- Volume 5B—Fresh fruits and vegetables
- Volume 6—Fruit juices
- Volume 7—Cereals, pulses (legumes), and derived products and vegetable proteins
- Volume 8—Fats and oils and related products
- Volume 9—Fish and fishery products
- Volume 10—Meat and meat products; soups and broths
- Volume 11—Sugars, cocoa products, and chocolate and miscellaneous products
- Volume 12—Milk and milk products
- Volume 13—Methods of analysis and sampling

Rule making and standardization activities are carried out through committee management system. Current general subject (also called horizontal committees) and commodity committees (vertical committees) are listed as under.

GENERAL COMMITTEES

- General Principles Committee (hosted by France)
- Food Labeling Committee (hosted by Canada)

- Methods of Analysis and Sampling Committee (hosted by Hungary)
- Food Hygiene Committee (hosted by the United States)
- Pesticide Residues Committee (hosted by the Netherlands)
- Food Additives and Contaminants Committee (hosted by the Netherlands)
- Import/Export Inspection and Certification Systems Committee (hosted by Australia)
- Nutrition and Foods for Special Dietary Uses Committee (hosted by Germany, a General Committee for the purpose of Nutrition)
- Residues of Veterinary Drugs in Food Committee (hosted by the United States).

COMMODITY COMMITTEES

- Committee on Fats and Oils (United Kingdom)
- Committee on Fish and Fishery Products (Norway)
- Committee on Milk and Milk Products (New Zealand)
- Committee on Fresh Fruits and Vegetables (Mexico)
- Committee on Cocoa Products and Chocolate (Switzerland)
- Committee on Sugars (United Kingdom)
- Committee on Processed Fruits and Vegetables (United States)
- Committee on Vegetable Proteins (Canada)
- Committee on Cereals, Pulses, and Legumes (United States)
- Committee on Processed Meat and Poultry Products (Denmark)
- Committee on Soups and Broths (Switzerland)
- Committee on Meat Hygiene (New Zealand)
- Committee on Natural Mineral Waters (Switzerland)

International food trade and associated food safety and consumer protection is well served by joint efforts of Codex, FAO, WHO, and ISO. This model is a classic example as to how international cooperation, leadership, and determination could serve as a binding force despite challenging technoeconomic realities of twenty-first century.

CONCLUSIONS

Dairy foods processing industry is showing growth and expansion with unique challenges especially in

food safety and quality management arena. Consumers expect safe and wholesome foods and it is the primary responsibility of processing industry to deliver safe and wholesome products. Experience has suggested that quality management is a complex challenge because of myriads of interactions involving technology, environment, microorganisms, and human interface. Despite impressive progress in science of food manufacturing, there remain significant limitations in commercial food safety/quality. Nevertheless, our understanding is improving as to how to address important quality and safety paradigms of today. Integration of science, technology, regulatory environment, and consumer demand has created food safety and quality society in the twenty-first century. Current body of knowledge suggests that quality control, quality assurance, and food safety challenges could be addressed by systematic approaches such as HACCP, TQM, Kaizen, and Six Sigma, to name just a few. It appears that HACCP system has found wider application and preference worldwide due to its practical benefits preventive nature, and associated cost benefits. Benefit of quality management are better realized when integration of process controls and all the people involved is optimized. Despite several limitations of current methodologies, its continuous use and positive learning curve would be expected to bring beneficial outcomes. If surge in risk analysis, predictive modeling, process control, and real-time detection capability is any indication; future of food safety and quality management is expected to be exciting and more subtle. It is reasonable to forecast based on cumulative knowledge that integration of new technology and human interface would be critical for cost effective, yet tangible food safety outcomes.

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23

Laboratory Analysis of Milk and Dairy Products

C. T. Deibel and R. H. Deibel

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INTRODUCTION

When looking at the microbial activity in dairy products, not only for shelf stability but also for organoleptic considerations and ability for fermentation to occur, of initial importance are evaluations of raw materials, in plant monitoring, and in some cases end-product testing. Bear in mind, “the result of any analysis is only as good as the ability to interpret the result.” The result is heavily dependent on the sampling procedure used; generally speaking, “there is no statistical relevance in a sample size of one.”

Throughout this chapter the terms standard and specification are mentioned and should not be used interchangeably. A standard is a quantity promulgated by local or federal regulatory agency. Violations of a standard may provoke legal action such as fines seizures, or recalls. In contrast, a specification defines the limits that an end user imposes on a product or sample. These qualitative or quantitative limits are usually defined in contracts between two parties, or are internally generated. A violation of a

specificatio may also result in rejection of a lot and return of the product.

Raw milk and pasteurized milk are analyzed at the laboratory level as much can be gleaned about the quality of the products before their use in subsequent manufacturing practices. Many factors will influence the quality of milk, such as animal husbandry, breeding practices, quality of feed materials, and medical care given to the herd, and these influence lend themselves prone to evaluation by testing methods. For instance, somatic cell counts are routinely used for testing the quality of raw milk. But several factors can influence this cell count such as relative health of the cow, "disease outbreak" in the herd (if milk is commingled), animal husbandry practices, and care of the herd.

The term "milk" is a lay term for "cow's milk." It is a colloidal suspension of hydrophobic fat molecules suspended in a hydrophilic carrier of water, sugars, protein, and minerals. It is a secretion from the mammary glands of mammalian animals, and may also contain beneficial proteins (antibodies) necessary for a newborn's survival. The main carbohydrate in milk is lactose; the main protein is casein (an allergen); and the major fats contain the fatty acids oleic and palmitic. In whole milk, most of the measurable acidity comes from the casein content and to a lesser degree the phosphate content (Deibel et al., 2005). An important nuance to be considered is the relationship between pH and titratable acidity: pH measures the strength of the acids in a solution, whereas titratable acidity measures the total amount of acids in a given solution.

It should be noted that the primary allergen in milk is casein. This poses a different consideration than for those individuals who suffer from a "lactose intolerance." A general definition of an "allergen" is any foreign molecule that induces an immune response in the host, specifically involvement of the immunoglobulin antibody IgE (Immunoglobulin E). Food-borne allergens are generally small protein chains, and are almost always the principle protein of the food constituent—casein in milk, gluten in wheat, and so forth. Those individuals who have a "sensitivity" to certain components such as lactose do not have any immune system involvement and therefore lactose is not classified as an allergen (Deibel et al., 2006). Lactose is a disaccharide composed of one molecule of beta-D-galactose and one molecule of beta-D-glucose, and would normally be broken down to its monosaccharide sugars by the lactase enzyme. In lactose-intolerant individuals, the body lacks the

ability to produce this enzyme, so the intact disaccharide is carried to the large intestine where it is broken down by the host body's natural microbial flora. Many bacteria have the ability to utilize lactose and readily metabolize it to its fermentative end products of CO₂ and acid. The copious amounts of gas that develop in the large intestine cause a certain amount of discomfort to the host, and the elevated acid production causes the body to compensate by flushing the intestines with added water. The overall after-effects of these two processes are self-evident.

Lactose-intolerant populations seem to follow genetic lineages especially in culture groups, as evident in the cuisines of choice. In most Asian populations, adults are lactose intolerant and therefore they do not routinely use dairy products, choosing instead to eat soy-based foods.

Of direct importance to the food industry, only allergens should be addressed at the plant level and on the product's packaging. Substances like lactose, which cause sensitivities to the population at large, do not currently have to be declared on the nutritional label of foods, although this may change in the future.

COMPOSITIONAL TESTS

FAT CONTENT

The fat content of milk and some other dairy products is economically important as it can determine price. In years past, it was the determinant; however, in current times other factors including nutritional considerations have come into play. The fat content in milk ranges from 2.5 to 6.0%, with a mean percentage of 4.0. The fat occurs as a globule encased in a membrane. The globules are suspended as an oil-in-water emulsion which can be disrupted by shaking or other mechanical means. The membrane encasing the fat globules consists of a lipid bilayer membrane similar to the apical membrane of epithelial cells.

Chemically, fats consist of a glycerol backbone to which three fatty acids are esterified (Fig. 23.1). The fatty acids vary in chain length and degree of unsaturation. The major fatty acids in milk are palmitic (25%), Oleic (20%), with steric and myristic making up about 10% each. Small amounts of butyric acid are also present (known to cause a "vomit-like" odor) and can contribute to a rancid flavor when it is cleaved from the glycerol backbone by the action of an inherent lipase enzyme in the milk, or by microbial activity.

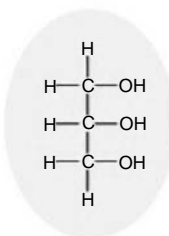
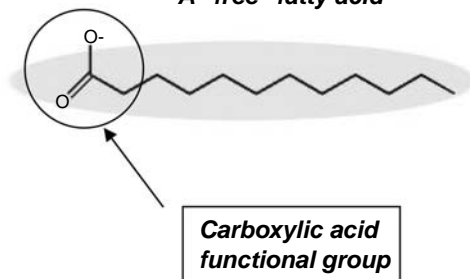
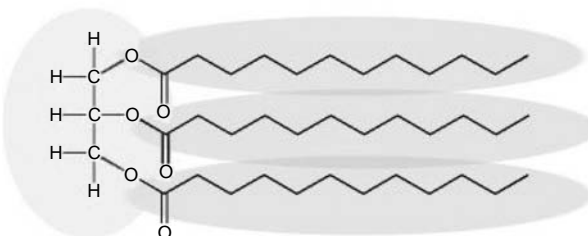
Glycerol**A "free" fatty acid****Triglyceride**

Figure 23.1. Chemical representation of a fatty acid. (Photo courtesy of the Olive Source [www.TheOliveSource.com].)

FAT DETERMINATIONS

In the United States, the most widely used total fat procedure for dairy products is the Mojonnier fat method. It is involved, tedious, and time-consuming; and, quite variable in the number of extractions and volumes of extractant (ethyl alcohol, ethyl ether, and petroleum ether). The latter depends on the product (Wehr and Frank, 2004) and the method requires a special "Mojonnier flask." Various modification of the Mojonnier method have been published including the Pennsylvania Roccal, Gerber Procedures, and "acid hydrolysis." (See Fig. 23.2).

An outline of the procedure is as follows:

1. To 10 g of milk in a Mojonnier flask add 1.5 mL of NH_4OH . The base neutralizes the sample and dissolves the protein.
2. After adding 10 mL of ethanol (to prevent gel formation), sequential additions of ethyl ether and petroleum ether (25 mL each) are added.
3. The sample and ether homogenate is vigorously mixed then allowed to stand until the layers separate.
4. The ether layer is decanted into a tared evaporating dish or beaker.
5. The above steps (2–4) are repeated up to three times, adding the decanted ether layers to the same evaporating dish or beaker.
6. In a hood, the ether solvent is evaporated over a moderately heated water bath using a hot plate, with the beakers under nitrogen gas to avoid ignition of the boiling solvent.
7. The evaporating dish or beaker is dried in a forced air oven at 100°C for 1 hour, cooled in a desiccator and weighed.
8. The evaporating dish is placed in a forced air oven until a steady weight is reached on the analytical balance.

Calculation:

$$\% \text{Fat} = 100 \times \left[(\text{weight of dish} + \text{fat}) - \text{weight of dish} \right] - \frac{\text{weight of blank}}{\text{weight of sample}}$$

The reagent blank is made with 10 g of water instead of milk.

In contrast to the essentially gravimetric Mojonnier method, the Babcock method is volumetric but expressed on a weight basis. It employs a special bottle with a graduated, constricted neck or stem (see Fig. 23.3).



Figure 23.2. Mojonnier flask picture. (Picture courtesy of Kimble Chase.)

Concentrated sulfuric acid is added to a weighted sample in a Babcock bottle (size and design vary with the product). The acid digests the proteins in the sample generating heat, while liberating the fats. The samples are lightly centrifuged (and constantly heated to keep the lipids in liquid form), followed by the addition of hot water to bring the fat layer into the graduated stem. The percent fat is read from the graduation on the stem. The method is rapid and simple but the temperature control of the sample and reagents is critical. The disadvantages are charring, particle contamination of the lipid layer, and the need for exactly calibrated bottles.

Several automatic methods are available. Some are derivatives of the standard Mojonnier method, but there are also rapid methods that are completely different. The turbidmetric approach is described in the Standard Methods for the Examination of Dairy



Figure 23.3. Babcock flask bottle. (Picture courtesy of Kimble Chase.)

Products (Wehr Frank, 2004). To the diluted sample, EDTA is added to eliminate protein particle interference. The sample is added to a Foss Milk Tester, or the equivalent, the light scattering is measured electronically and a direct readout is obtained. The procedure is rapid, accurate, and precise using a properly calibrated instrument.

Other common fat testing methods include the Soxhlet extraction method (see Fig. 23.4), with calculations being derived as “fat by difference” similar to the Mojonnier method, only the fat is extracted by an alcohol solvent over heat.

Methods

Fat Sample Preparation AOAC 920.125 (2006)

Fat by Modifie Mojonnier AOAC 933.05 (2006)

Fat by Soxhlet AOAC 963.15 (2006)

Fat by Babcock AOAC 974.09 (2006)

(Other methods for Fat determinations are available, but the reader is cautioned only to use those analyses that have been validated against their specific sample matrix.)



Figure 23.4. Picture of a Soxhlet apparatus for fat determinations. (Picture courtesy of Kimble Chase.)

FREE FATTY ACIDS

Fatty acids vary in length but most are carboxylic acids with long unbranched “tails” or “chains” of carbon molecules. They are classified generically as either being “saturated” or “unsaturated,” as identified by the hydrogen atoms on the carbon chain. If the carbon atoms are completely filled with hydrogen atoms [with the exception of the carboxylic acid ($-\text{COOH}$) functional group], then they are considered “saturated.” Unsaturated fatty acids have at least one double bond between adjacent carbon groups, such that the normal “ $-\text{CH}_2-\text{CH}_2-$ ” of a saturated chain has a “ $-\text{CH}=\text{CH}-$ ” (called an alkene functional group).

Fatty acids can be either bound or unbound to other molecules, mainly triglycerides. In their unbound state, they are called “free fatty acids (FFA).” They are commonly used in the food industry as a

measure of rancidity, as they are prone to normal acid–base reactions of the carboxyl function group or “auto-oxidation.” The fatty acid will naturally break down into its constituent parts, mainly hydrocarbons, ketones, aldehydes, and smaller amounts of alcohols. In their pure forms, or in products with a high fat content, these oils and fats are often treated with chelating agents, such as citric acid, to retard this auto-oxidation process.

The quickest and easiest way to test for FFA is by direct acid–base titration using an ether–fat extraction, and endpoint determination by a phenolphthalein color change. The main drawback to this method is that the endpoint can be difficult to determine just by visual means. Automatic systems that can determine the pK_a endpoint are a much more reliable means, but they are often costly. However, this cost difference can often be offset by the decreased technician time that would be spent on the manual titration process. This obviously is only feasible for laboratories with a larger throughput of samples.

The FFA content is calculated as oleic acid. To the liquid fat sample, an ether extraction is added, neutralized ethanol-containing phenolphthalein. The sample is titrated with standardized sodium hydroxide (Wehr and Frank, 2004).

Calculation:

$$\begin{aligned} \% \text{Free Fatty Acid} \\ = \frac{\text{mL NaOH} \times \text{Normality of NaOH} \times 28.2}{\text{weight of sample}} \end{aligned}$$

Methods

Free Fatty Acid by Titration AOAC 940.28 (2006) (Other methods for free fatty acids are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

PEROXIDE VALUE

In the initial, or primary, period of lipid oxidation, the peroxides or lipoperoxides are formed. The free radicals react with iodide forming free iodine which is titrated with standardized sodium thiosulfate using a starch indicator. The value is expressed as milliequivalents of peroxide per kilogram of fat.

Calculation:

$$\text{Peroxide Value} = \frac{\text{mL S}_2\text{O}_3^{(-2)} \times \text{Normality} \times 1000}{\text{grams sample}}$$

Maloaldehyde can accumulate in the terminal or secondary phase of lipid oxidation. The aldehyde reacts

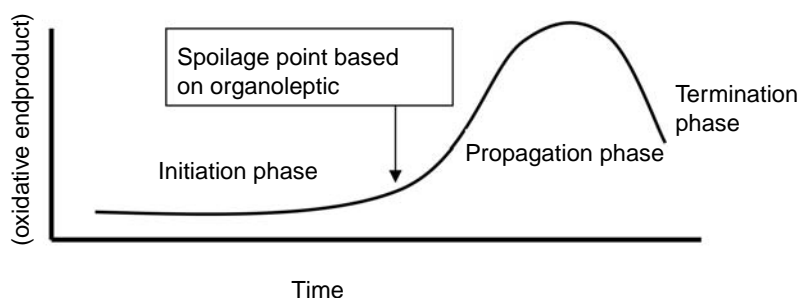


Figure 23.5. Graphical representation of oxidative rancidity. (Courtesy of Deibel Laboratories, Inc.)

with thiobarbituric acid to form a colored compound. The reaction is not specific for malonaldehyde and interfering compounds can also produce color. The test is not used as frequently as the peroxide value in dairy products.

Atmospheric conditions such as heat, light, storage methods, transition metals, chemical oxidizers, and enzymes such as lipoxygenase can contribute to the rancidity of food products. Research findings indicate hydrolytic and oxidative rancidity are two forms of lipid oxidation. Oxidative rancidity has initiation, propagation, and termination phases. Under normal conditions, a fat is composed of three fatty acid chains plus a molecule of glycerol. During the initiation phase, oxygen disrupts a double bond in the fatty acid chain and forms a molecule with an uneven distribution of electrons hence a free radical. In search of stability, the free radical reacts with another molecule of oxygen creating a chain reaction effect which leads to the propagation phase characterized as a proliferation of free radicals. The byproducts of this phase are hydroperoxides which are expressed in milliequivalents. During the last phase, hydroperoxides reach a certain level and decomposition occurs. The degradation of hydroperoxides leads to the formation of nonradicals such as aldehydes and ketones which are byproducts of the terminal phase.

Hydrolytic rancidity appears to be the least understood lipid pathway. However, it is understood that triglycerides are broken down via hydrolysis of ester linkages by lipase enzymes. This reaction in turn leads to the formation of FFA. The contributing factors of both oxidative and hydrolytic pathways contribute to the taste, smell, and texture of the product. Theoretically, lipid oxidation can occur as soon as it leaves the production line. Milliequivalents per kilogram of peroxide are tested using iodide and a titration. FFA is also tested using a titration. Both

results give some indication to how much oxidation has occurred between the time the product was manufactured and the time it reaches the laboratory (see Fig. 23.5).

Methods

Peroxide Value by Titration AOAC 965.33 (2006)
(Other methods for Peroxide Value detection are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

TOTAL SOLIDS

The water content of a food exists in three forms. Free water, absorbed water (in cells and bound to proteins), and chemically bound (water of hydration) water. There are many methods for determining the moisture content of a food. The form of water in the food, its concentration, the desired accuracy and precision of the analysis, and the analytical time to result are all important considerations to take into account. Sample collection is important as moisture can be lost or gained if the proper sampling techniques and collection containers are not used. The headspace in the container must be kept to a minimum. The moisture content of a food is important to the manufacturer as it is subject to a standard of identity, a customer's specification, the shelf stability of the product (dried and concentrated milks), and in some instances the quality control practices in the plant.

After the moisture is removed, a value referred to as total solids is obtained.

Calculation:

$$\% \text{Total Solids} = 100\% - \% \text{Moisture}$$

Another "total value" used in the dairy industry is the total solids-not-fat.

Calculation:

$$\% \text{Total Solids} = 100\% - (\% \text{Moisture} + \% \text{Fat})$$

Methods

Total Solids in Ice Cream AOAC 941.08 (2006)

Solids (Total) in Milk AOAC 990.19 (2006)

Solids-Not-Fat in Milk AOAC 990.21 (2006)

(Other methods for Total Solids are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

MOISTURE

Moisture values find utility in product specification and standards of identity. Percent moisture determinations in the dairy industry are rather product specific when selecting the method. The most common procedure employs a vacuum oven drying at 100°C for about 5 hours. Cheeses, dried milks, casein powders, and dry ice cream mixes fall into this category. For dried whey and whey products, the temperature is reduced to 65°C and the drying period extended to 16 hours. Fluid milks require a partial drying (usually over a steam bath) prior to vacuum drying.

The general methodology involves a homogenization in a mechanical blender, weighing the sample on a tared analytical balance in a disposable aluminum pan with a cover (to avoid splattering), heating, desiccation, and re-weighing. With some products, other procedures can be used, such as the forced-draft-oven method, the microwave oven, or other multi-component procedures (lactometric or infrared). Two excellent sources for specific product methodology are AOAC (2006) and Standard Methods for the Examination of Dairy Products (Wehr and Frank, 2004).

ADDED WATER

When a solute is added to water the boiling point is increased and the freezing point is decreased. In the latter instance, this principle is utilized to detect water that has been added to milk to increase its volume. Thus, if water is added to milk, the water-soluble substances (primarily inorganic salts and lactose) are diluted and the freezing point is increased over the normal average of 0.517°C. The AOAC (2006) has standardized this procedure using a cryoscope and in this method, some tolerances have been incorporated to account for a variety of factors that can influence the values obtained.

Methods

Added Water (Milk) by Cryoscope AOAC 980.15 (2006)

Added Water (Milk) by Refractive Index AOAC 948.10 (2006)

(Other methods for %Moisture are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

WATER CONTROL IN CERTAIN DAIRY PLANTS

Bacteria, especially the pathogens and common food-spoilage types, require relatively large concentrations of free water for growth. Indeed, biologists of yesteryear considered bacteria as “water plants.” Perishable foods have a free-water phase and the greater the free water concentration the greater the potential for spoilage (or decreased shelf life). Enhanced shelf life can be affected by increasing the solute concentration (usually by salting or sugaring) the water phase of the food. Alternatively, dehydration can be employed to achieve the same result in some products.

In a food plant, there will always be nutrients for the microorganisms (the food product being produced), and since bacteria are ubiquitous, they will always be represented in the food plant. The only ability for control revolves around the sagacious and judicious use of water in the facility. We can compare “Microbial Growth” to that of a fire. In nature, there will always be “nutrients” for a fire which includes the kindling temperature, oxygen, and a fuel. In much the same way, microbial growth is linked to water, organic matter, and the incidence of microbes. Thus, in certain dairy plants (such as those for dried products) the control of water (roof leaks, valve and pipe leaks, condensation, and wet-cleaning practices) is critical (Fig. 23.6).

WATER ACTIVITY

Assume that a food is placed into a closed container. After a brief time, the water in the food comes into equilibrium with the headspace in the container. For the purpose of illustration, assume that as many molecules of water escape from the food and an equivalent number of water molecules reenter the water phase creating a vapor pressure (P_o) in the container. Assume that another container has the same food product but sugar or salt has been added, thus

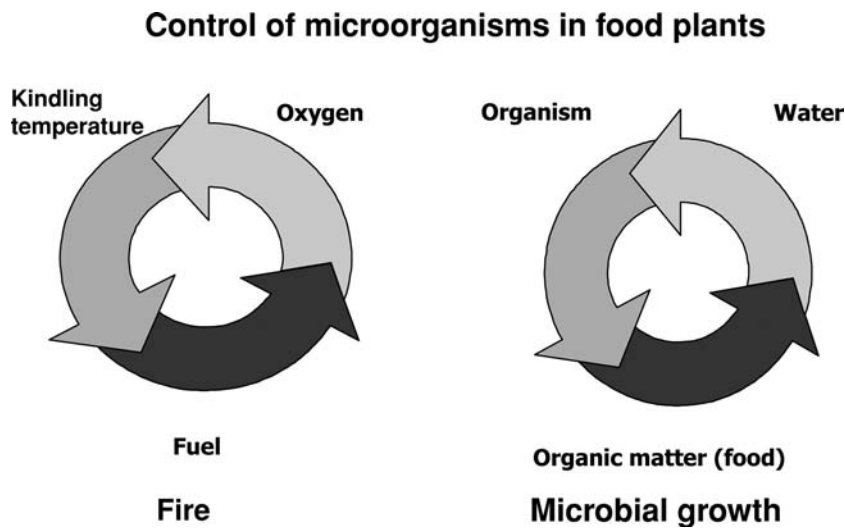


Figure 23.6. Diagram of bacterial growth in food plants. (Courtesy of Deibel Laboratories, Inc.)

binding some water and diminishing the vapor pressure (P_s) (Fig. 23.7).

Thus, the vapor pressure of $P_o > P_s$ and the ratio of pressures define the water activity (a_w).

$$a_w = \frac{P_s}{P_o}$$

For pure water, $P_o = P_s$; the ratio is unity and the value of a_w for a food is less than 1.0.

Microorganisms cannot grow in pure water so their maximum a_w approaches 1.0. All microorganisms

have a minimum a_w for growth and their value differs from a lower value for survival. Complete removal of water from a microbial population or lyophilization enhances survival and provides a means of stocking cultures for protracted periods.

Some of the a_w values for common foods and minimum growth values for microorganisms encountered in dairy products are presented in Figure 23.8. Generally, yeasts and molds are more tolerant to low a_w values for growth. Some of the latter microorganisms, called osmophiles, absolutely require high-solute concentrations for both growth and survival; higher a_w values can kill these organisms fairly readily as they are affected by “osmotic shock” and their cell membranes collapse.

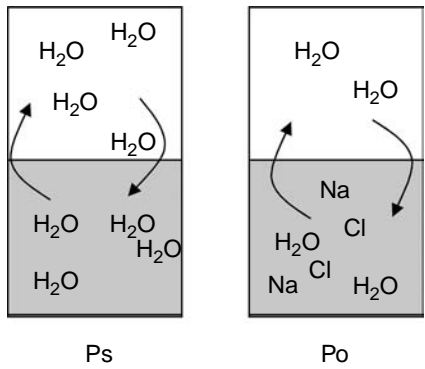


Figure 23.7. Diagram of vapor pressure in pure water in contrast to vapor pressure of a high-solute solution. (Courtesy of Deibel Laboratories, Inc.)

Other Water Activity Instruments (Hygrometers and Dew Point)

Electric hygrometers and dew point instruments are economically available to estimate the a_w . There are many instruments available which give rapid and fairly accurate results. Generally, these instruments have minimal need for calibration; however, cost of the instrument may be an issue. For laboratories with low throughput, these may not prove to be economically viable options for a_w determinations.

Methods
Water Activity AOAC 978.18 (2006)

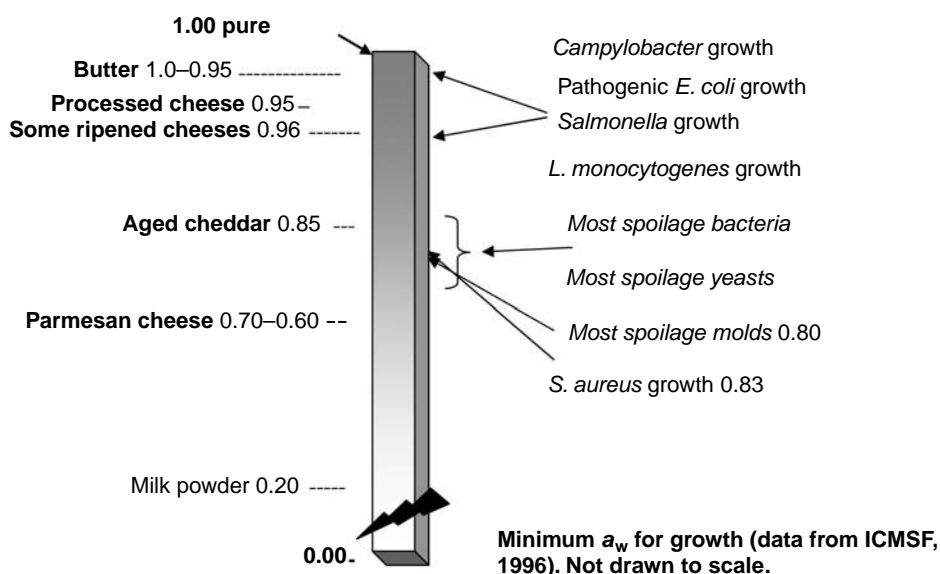


Figure 23.8. Water activity (a_w) of dairy products and microbial growth. (Diagram courtesy of Deibel Laboratories, Inc.)

(Other methods for Water Activity are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

PROTEIN DETERMINATIONS IN DAIRY PRODUCTS

All plant and animal cells have structural and biologically functional proteins. The building blocks of these proteins consist of 20 alpha amino acids [R-CH-NH₂-COOH] that are linked through a peptide bridge. Proteins are immensely interesting as they have the ability to form very complex molecules, and are typically represented in four different forms: primary, secondary, tertiary, and quaternary (see Fig. 23.9).

Milk proteins, such as casein, lactalbumin, or lactoglobulin, contain varying amounts of amino nitrogen and aromatic amino acids. Analytical procedures for protein content in dairy foods involve the determination of nitrogen (i.e., Kjeldahl), dye-binding (Amido Black), and spectroscopic procedures. The most commonly used method is the Kjeldahl because of a relatively short time to result and low-cost. The result, %Protein, expressed as %Kjeldahl Nitrogen. Several variations on the central testing scheme have increased the accuracy of the analytical procedure.

“Rapid Testing” systems, though still married to the generally Kjeldahl scheme, pose an economically attractive alternative. This is due to decreased solvent usage as well as increased speed. However, the methods are still prone to a healthy amount of “acceptable error.”

Generalized Kjeldahl method is given as follows:

1. Homogenize the sample
2. Acid digestion with sulfuric acid

$$\text{Protein} + \text{H}_2\text{SO}_4 (\text{Heat, Catalyst}) \rightarrow (\text{NH}_4)_2\text{SO}_4$$
3. Neutralization using a mixture of sodium hydroxide (plus sodium thiosulfate to promote ammonia distillation)

$$(\text{NH}_4)_2\text{SO}_4 + 2\text{NaOH} \rightarrow 2\text{NH}_3 + \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}$$
4. Distillation of ammonia into a solution of standard HCl

$$\text{NH}_3 + \text{HCl} \rightarrow \text{NH}_4\text{Cl}$$
5. Titration of excess HCl with standardized NaOH
6. Calculation

Throughout the years variations in just about every step have been published but the reader is referred to the AOAC for the standard method, which is outlined above.

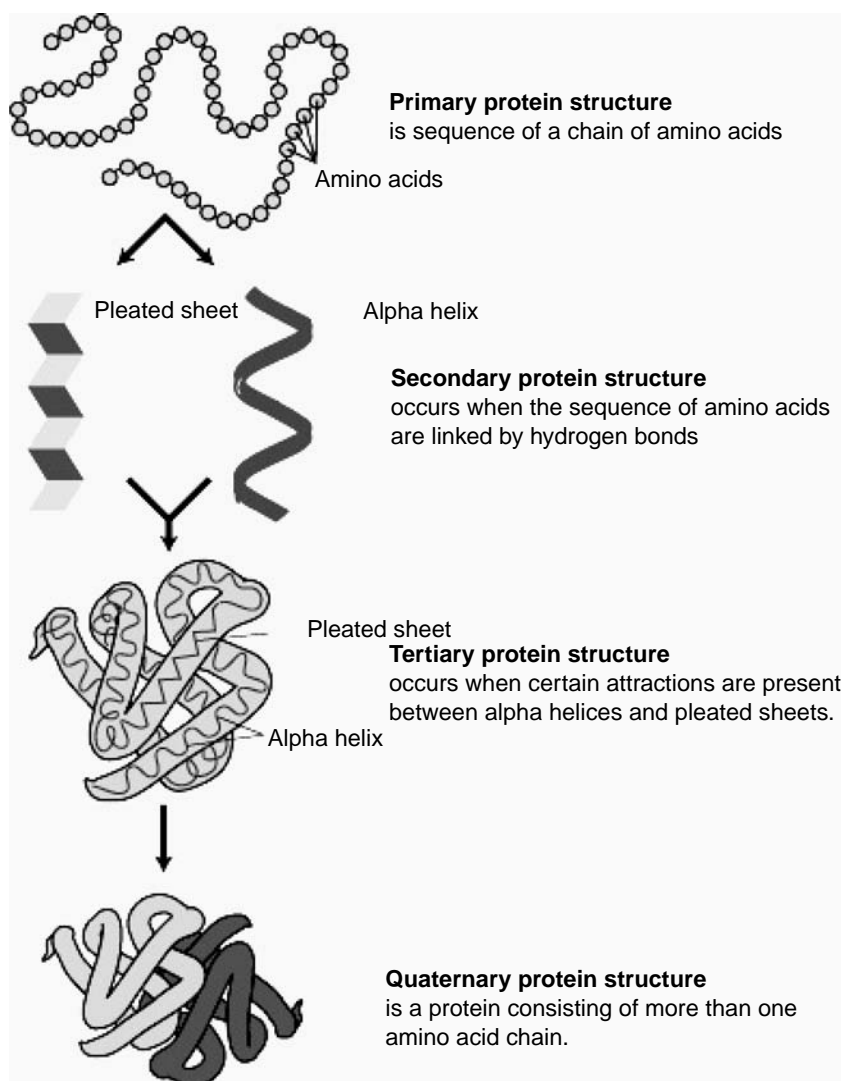


Figure 23.9. Protein shapes. Graphics courtesy of National Human Genome Research Institute (NHGRI) (<http://www.nhgri.nih.gov/DIR/VIP/>) by artist Darryl Leja.

Generally, proteins consist of about 16% nitrogen. Therefore, the conversion factor is given by:

$$\frac{\%N}{0.16} = \%Protein$$

$$\text{or } \frac{100}{0.16} = 6.25$$

So, %Nitrogen can be calculated as follows:

$$\%N \times 6.25 = \%Protein$$

Milk proteins contain a slightly lower amount of nitrogen in their proteins, and a factor of 6.38 is used in the calculation.

A micro Kjeldahl procedure and various rapid methods have been published. Some find utility in quality control of product; however, regulatory agencies, tests for standards of identity (and/or nutritional labeling), and trade contracts require the official AOAC method. This method is relatively simple to perform, it is applicable to a wide variety of dairy

foods, and it is relatively inexpensive. The main costs involve “Technician Time” and the use of expensive solvents. Many rapid methods are automated, with lower manual time spent on the procedure; and, also these methods use smaller quantities of solvent. However, extreme care must be exercised in handling the corrosive reagents and the test must be conducted in a chemical or spill-proof fume hood. In some cases, laboratories have a dedicated, explosion-proof room to perform these tests.

Another error of the test is that it measures total nitrogen, not just that which is found in proteins. Phospholipids, and other cellular constituents, contain measurable amounts of nitrogen. Foods containing relatively large amounts of these nonprotein molecules should have this taken into account.

Methods

Protein content by Kjeldahl AOAC 939.02 (2006)
(Other methods for % Protein are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

LACTOSE IN DAIRY PRODUCTS

Lactose is the principal sugar found in most of all mammalian milk, with only two to three exceptions, which are not germane to this discussion (i.e., the sea lion and the duck-billed platypus do not produce noticeable lactose in their milk). It is a disaccharide composed of the monosaccharides L-glucose and L-galactose. Dairy starter cultures of yesteryear split the lactose and fermented the glucose while the galactose would accumulate in the product. Today’s starters can be selected (mutated) to ferment both monosaccharides.

Lactose lacks the sweetening power of the plant sugar sucrose. In recent times, confectioners have added substantial amounts of lactose to certain products as it enhances stability and improves yield. This has provided the dairy industry with the opportunity to utilize another fraction of milk aside from dried whey, casein, butter fat, and other commercial fractions.

The chemical analyses to quantitate the lactose are quite involved and tedious and require sophisticated equipment. Polarimetric, gravimetric, infrared, enzymatic, and high performance liquid chromatography (HPLC) methods have been greatly advanced in the past 10 years. The polarimetric and HPLC methods are outlined in Standard Methods for the Examination

of Dairy Products. The former is relatively straightforward; however, a polarimeter is required and the disposal of the mercury used in the assay requires special handling and attention. The HPLC procedures are indeed involved, but are the standard in the industry as there are no special handling or disposal concerns beyond those of any normal “wet chemistry” laboratory. HPLC assays offer the added benefit of testing for additional sugar fractions in the sample, such as dextrose, fructose, sucrose, and maltose.

HPLC Procedure

A 5.00-g sample is dissolved or extracted by stirring, heating, and/or shaking in water. The solution used for analysis is usually a 5% solution or suspension of sample, and contains 10% acetonitrile in 100 mL of aqueous solution. The extract is filtered chromatographed by HPLC, and the sugars identified and quantitated by comparison of the sample chromatogram with that of the sugar standards. Spiking of some samples may be useful in identifying peaks. Standard solutions contain fructose, dextrose, maltose, lactose, and sucrose. Single standards or combination standards may be used.

Polarimetric Procedure

Into 100 mL and 200 mL volumetric flasks weigh 65.8 ± 0.05 g of sample. After adding 30 mL of mercuric iodide solution to each flask (33.2 g KI, 13.5 g HgCl_2 , 200 mL glacial acetic acid, and 640 mL H_2O), 5 and 15 mL of a 5% solution of phosphotungstic acid is added and each flask is diluted to volume. The flasks are shaken periodically over a 15 minutes period and then filtered. The samples are then polarized using 200 mm and 400 mm polarimetric tubes. The percent lactose is calculated as follows:

$$\% \text{Lactose} = R_{100} - \frac{[2(R_{100} - R_{200})]}{2}$$

Methods

Sugar Profile by HPLC AOAC 980.13 (2006)

Lactose in Process Cheese by Polarimeter AOAC 930.32 (2006)

(Other methods for Lactose detection are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

ACIDITY IN DAIRY PRODUCTS

Titrateable acidity can be a very valuable in-plant means of indirectly measuring the extent of bacterial growth (fermentation) of a cultured dairy product. Measuring the titrateable acidity can answer two important questions for the finished material: "Has the starter culture grown?" and "What is the extent of fermentation?" In cheese manufacturing, it is used mainly as the endpoint determination for when to "cut the curd."

Acidity alone is not a good measurement of milk quality, as the "number of bacteria must increase to several millions per milliliter before there is a measurable increase in acidity." It can be of value when compared to the historical quality of milk from a single supplier, but in and of itself it does not provide a good diagnostic tool. This is due mainly to the inherent buffering capacity of milk. At the laboratory level, milk is often used as a "preenrichment" for culturing Gram-negative bacteria, such as *Salmonella*. With constituent components such as proteins (casein) and phosphates, aiding in its ability to absorb and neutralize larger quantities of acid, it provides a superior media for resuscitating sublethally injured cells, such as those commonly found in heat-treated, manufactured products.

Additionally, the measurable acidity varies greatly from cow-to-cow and from herd-to-herd (0.10–0.26% and 0.18–0.23%, respectively) (<http://drink.edudairychem5.htm>), making any sort of definitive determination on this testing extremely difficult

Methods

Acidity in Cheese AOAC 920.124 (2006)

Acidity of Milk AOAC 947.05 (2006)

(Other methods for Acidity are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

PH

pH is a measurement of the strength of an acid, by measurement of the hydronium ion concentration [H_3O^+]. It is a very easy test in the laboratory but more importantly for in-plant monitoring. There are many in-line monitoring probes that can be used that also provide for unfettered access to the product in closed systems without having to risk exposure to the environment. "Hydriion slips" of pH paper can also be used as they give real-time colorimetric

determinations, although they are generally less sensitive than those obtained by electronic means.

Methods

pH (Wehr and Frank, 2004)

(Other methods for pH detection are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

CHEMICAL TESTS FOR FLAVORFUL SUBSTANCES

Aside from FFA, lipid oxidation products, and moisture, the predominant organoleptic constituent in dairy products results from the microbial fermentation of citric acid ending in diacetyl formation. This end product imbues the sample with a buttery flavor, adding to the flavor profile of many cheeses and yogurts. This is the contributing flavor compound in butter. In fact, dairy starter cultures are screened for their ability to produce diacetyl, as a necessary prerequisite for their use in the industry.

Milk contains a relatively small amount of this flavor contributing acid and the concentration ranges from 0.13 to 0.18%. The citrate is fermented to pyruvate which is the source of an active two carbon intermediate. The latter dimerizes forming acetylmethylcarbinol (AMC). Under slightly aerobic condition, AMC is oxidized to form diacetyl. The diacetyl can be measured chemically but most often it is evaluated by a sensory panel with other flavor attributes.

Under anaerobic conditions, the AMC can be reduced to 2–3 butylene glycol (2–3 BG) which is flavorless (see Fig. 23.10).

MILK TESTING

RAW AND PASTEURIZED MILKS

Fresh raw milk from healthy animals contains small amounts of bacteria (characteristically about 1,000/mL). As the milk is collected at the farm level and transported and stored at the processing plant, the count increases in this high-moisture product. The standard for grade A milk from a single producer for pasteurized milk is 100,000 bacteria/mL and for commingled milk, it is 300,000 bacteria/mL. The standards for manufacturing milk are more lenient; 500,000/mL for grade 1 and 1,000,000/mL for grade 2. The latter milks are primarily used in cheese manufacturing.

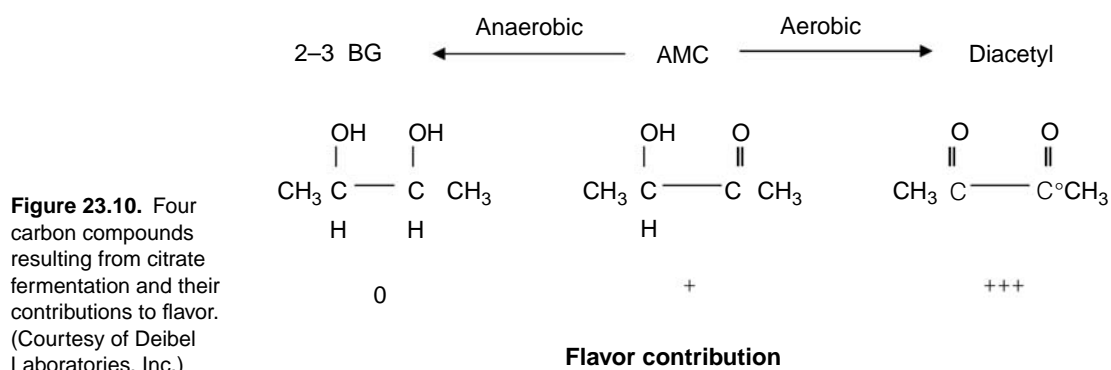


Figure 23.10. Four carbon compounds resulting from citrate fermentation and their contributions to flavor. (Courtesy of Deibel Laboratories, Inc.)

Several so-called “cow side” tests are available to screen for abnormal or mastitic milk. The California Mastitis Test employs a detergent to lyse the somatic cells thus liberating DNA from the cell’s nuclei. Various degrees of positivity are subjectively noted and the mastitic animal can be treated.

An inflamed udder produces less lactose than normal in the milk, and osmolyte serum salts are physiologically added. These salts can be qualitatively estimated using a hand-held conductivity meter and a diagnosis made.

A number of official methods are available for in-laboratory, direct microscopic, somatic cell, and bacterial counts. All are somewhat tedious and involved, so the analyst should have some experience in the testing methodology. Additionally, some states require an analyst to become certified before they are able to perform these tests. These procedures confirm the screening tests that were previously estimated for somatic cells. The direct bacterial counts can reflect sanitary conditions and have utility in this respect.

To a microscope slide having a circular area of 1.0 cm² that is clearly defined (slides are commercially available), a 0.01 mL portion of the sample is added using a special syringe. The sample is spread, dried at 40–45°C, and stained. Using a specially calibrated microscope and a single strip observation field (calibrations and calculations are involved), the number of somatic and bacterial clumps is estimated. There are many factors that affect the counts; and, at best, it is an estimator. Results reflecting counts greater than 300,000 somatic cells per mL are indicative of a mastitic infection.

Optical (automated) methods are AOAC approved for somatic cells (i.e., Fossomatic) (AOAC, 2006). The equipment is expensive and requires strict maintenance, but rapidity of results as well as facilitation of a large number of samples is advantageous.

The high-temperature short-time pasteurization procedure (71.6°C for 15 seconds) is most commonly used in the United States. This involves heating every portion of the milk such that the most heat-resistant pathogen is killed. Streamlined production obviates the low-temperature long-holding (62.8°C for 30 minutes) vat pasteurization process except for specialty items like some ice creams and specialty cheeses. The standards for grade A pasteurized milk in packaged products are a plate count of 20,000 bacteria/mL or less and a coliform count of 10/mL.

A unique milk containing enzyme, an alkaline phosphatase, has a heat sensitivity that closely parallels the requisite thermal death time for pathogens in milk. This is the index of adequate pasteurization. Several substrates to detect phosphatase activity have been used but the most popular is phenolphthalein monophosphate. The latter is hydrolyzed liberating phenolphthalein which can be quantitatively estimated colorimetrically or by a variety of methods most of which are AOAC approved. In-plant pasteurization is closely monitored by regulatory agencies.

If milk or cream products are held at slightly elevated temperatures for extended periods of time, a reactivation of the phosphatase enzyme can occur. Tests have been developed to detect this possibility. Also, a heat-stable enzyme can be produced by certain bacteria occurring in milk. Despite these relatively rare shortcomings, alkaline phosphatase

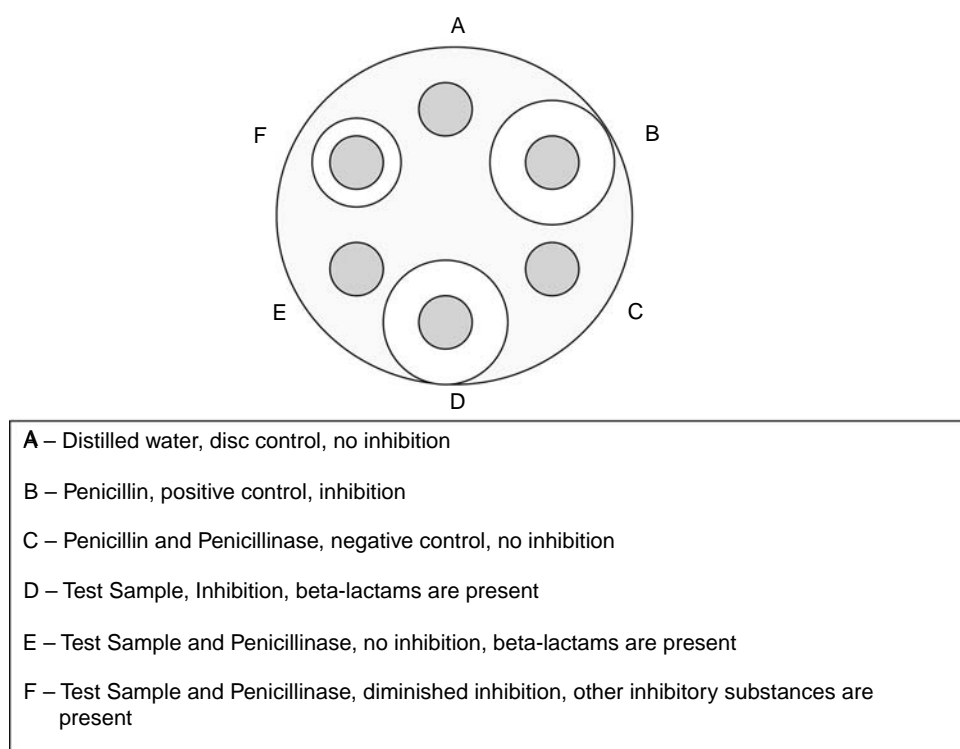


Figure 23.11. Diagrammatic representation of *B. stearothermophilus* response in an agar diffusion test for beta-lactams. (Courtesy of Deibel Laboratories, Inc.)

destruction is still used as the index of proper pasteurization.

INHIBITORY SUBSTANCES IN MILK

The major group of inhibitory substances in milk is antibiotics—especially beta-lactam antibiotics (penicillin and related compounds). Other broad-spectrum antibiotics are encountered less frequently. Antibiotics are used extensively on the farm to treat mastitis and respiratory infections. During and after treatment (antibiotic specific) the milk should be discarded, as the antibiotic will pass into the udder and into the milk. Occasionally, some of this milk may be incorporated in the general supply where it can inhibit starter cultures or cause adverse reactions in some susceptible individuals that are allergic to the antibiotic.

There is a plethora of assays for detecting and quantifying antibiotics in milk, especially the beta-lactams. Biological (zone inhibition using pa-

per discs), immunological (ELISA), and chemical (HPLC) methods have been approved by regulatory agencies and AOAC. The disc-diffusion procedure using the thermophilic bacterium *Bacillus stearothermophilus* has been AOAC approved for both qualitative and quantitative analysis for beta-lactams. In the procedure, an agar growth medium is inoculated with a standardized, commercially available spore preparation, and test and control solutions are added to the filter paper discs. The latter are placed on the inoculated agar medium and incubated at 64°C for approximately 3 hours. The spore load, the depth of the agar, and the temperature of incubation are critical and must be carefully controlled. The zones of inhibition are measured with a vernier caliper and interpreted as described in Figure 23.11.

Methods

Beta-Lactam Antibiotics in Milk AOAC 982.16 (2006).

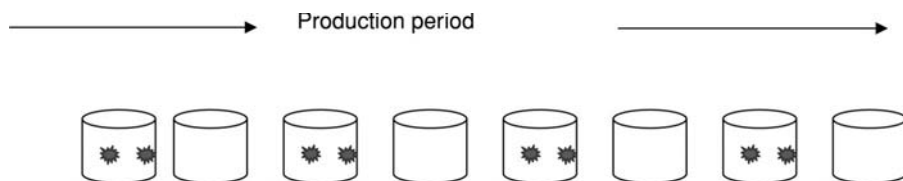


Figure 23.12. Random distribution of a contaminant in a production run. (Figure courtesy of Deibel Laboratories, Inc.)

MICROBIAL ECOLOGY IN FOODS IN RELATION TO PROCESSING

The following is taken from the Deibel Laboratories Client Education manual.

RANDOM DISTRIBUTION OF MICROBIAL CONTAMINANTS IN A LOT OF PRODUCTION

Microorganisms are rarely homogeneously (or randomly) distributed in food products or in the environment for that matter. A possible exception might exist in extremely well-mixed, fluid products; but generally, microorganisms naturally occur in a heterogeneous (or nonrandom) distribution. To further complicate the matter, microorganisms are prone to clumping or forming aggregates by virtue of excreted substances (i.e., “slime”) or structures on the bacterial cell walls that actually help them adhere to their surroundings. The clump can contain variable numbers of viable cells; mixing of the sample tends to break up the clump and will alter the quantitative [i.e., aerobic plate count (APC) or most probable number (MPN) counts] results.

If microorganisms were homogeneously (or randomly) distributed in a given food production period, the following graphic representation would be

expected. This type of microbial contamination is considered independent of time since random sampling of the entire lot will yield an equal chance of finding the microbial contamination (see Fig. 23.12).

NONRANDOM DISTRIBUTION OF MICROBIAL CONTAMINANTS IN A LOT OF PRODUCTION

Random distribution, as mentioned previously, is rarely encountered. The nonrandom distribution occurs much more frequently and is a result of many different factors and production situations. For example, a pattern such as the following could occur as a result of improper sanitation of the production equipment.

Here, at startup of production, the microbial contamination is the highest. As the production cycle increases, the product actually cleans the contaminated equipment. Therefore, as the production cycle increases, the microbial contamination is brought down to undetectable levels (see Fig. 23.13).

NONRANDOM DISTRIBUTION: MICROBIAL BUILDUP

This situation might reflect a buildup on a conveyor or in a slicing machine, such that as the production cycle

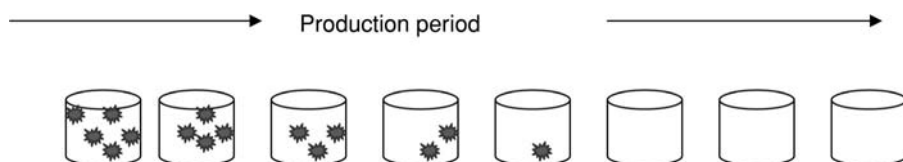


Figure 23.13. Nonrandom distribution of a contaminant in a production run. (Figure courtesy of Deibel Laboratories, Inc.)

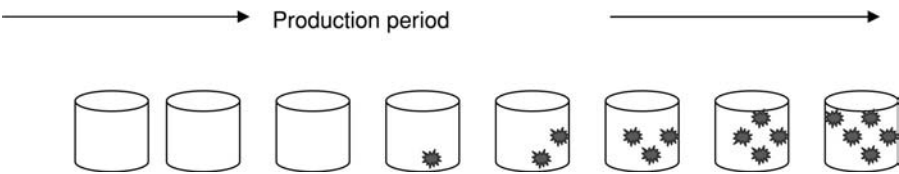


Figure 23.14. Nonrandom distribution—microbial buildup. (Figure courtesy of Deibel Laboratories, Inc.)

increases, the microbial contamination increases. A microbial buildup during the production cycle would be represented as given in Figure 23.14):

**NONRANDOM DISTRIBUTION:
INTRODUCTION OF MICROBIAL
CONTAMINATION**

This pattern, described below, can result from the introduction of a contaminated ingredient (i.e., a new lot), the introduction of a different batch of rework, or line breakdown and the restart of production, or in a seal or tube break (see Fig. 23.15).

The above scenarios should aid in the explanation of the nonrandom distribution of microorganisms in a lot of production, but note that these are not the only types of nonrandom distribution. When a retest analysis does not correlate with the initial test analysis, other factors or situations should be considered. The importance of obtaining a representative sample for the initial test and retest analysis cannot be overstressed (see “sampling” below for a better explanation of this important consideration).

**NONRANDOM DISTRIBUTION: ORGANISM
DIE-OFF**

Another type of microbiological concern, as opposed to either equipment failure, environmental concerns,

or improper sanitation, occurs after the microbes have entered into the product. Although the above considerations have focused on how a microbiological contaminant can enter into the production cycle, it is important to consider *how the microorganisms are affected by the product*. For example, the diagram below is a single lot of production viewed at three different time periods, Day 0, Day 7, and Day 14. During storage, the microbial contaminants have actually decreased in number (died off). Many factors can affect an organism’s die-off in a product, for instance, antimicrobial substances, pH, salt concentrations, a_w , and storage temperature to name a few.

Microorganisms vary greatly in their resistance to die-off. For example, *Salmonella* can persist in dried milk or chocolate for extended periods. In contrast, *E. coli* and *S. aureus* die-off relatively rapidly. In several instances, it has been observed that an *E. coli* contamination in a chocolate product, after retest by a standard FDA BAM Category II sampling scheme (i.e., 30 random 25 g samples, composited into two 375 g preenrichments), only negative results were obtained. Originally, several confirmed positive *E. coli* results were obtained for that production lot, but the retest was requested several weeks after receipt of the original positive results (see Fig. 23.16).

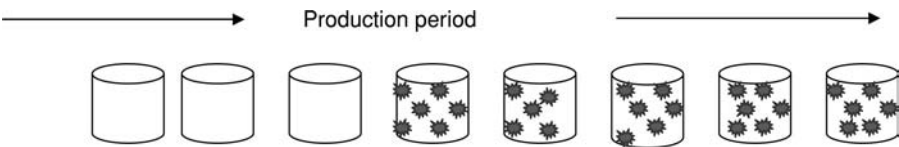


Figure 23.15. Nonrandom distribution—introduction of a contaminant into the production run. (Figure courtesy of Deibel Laboratories, Inc.)

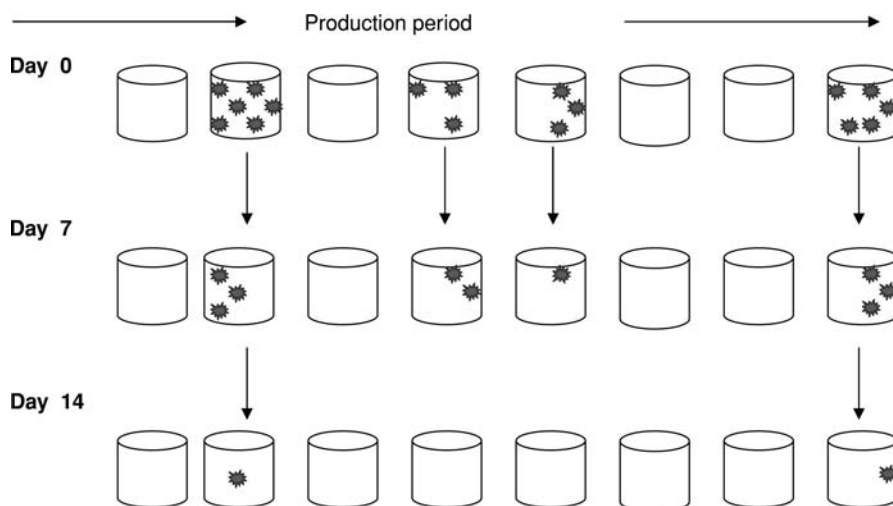


Figure 23.16. Organism die off. (Figure courtesy of Deibel Laboratories, Inc.)

To avoid issues of organism die-off, retesting *must* involve an expanded sampling plan and a rapid request for a retest. It is recommended that the laboratories be allowed to automatically retest any out-of-specification result. This saves the time requesting a retest and speeds the release of product.

NONRANDOM DISTRIBUTION: ORGANISM GROWTH (DIMINISHED SHELF LIFE OF THE PRODUCT)

There are times when a retest result is much higher than the original test result. This can be seen in the example below where the microbial contaminant has actually grown in a product during storage or holding. This example could reflect improper holding temperatures (i.e., a malfunctioning refrigerator in a truck or storage warehouse) or perhaps normal growth during storage. Both examples have been seen in such products as milk, cheese, yogurts, and other products. This example shows a diminished shelf life for the product, although there are *many* factors that can affect a diminished shelf life (see Fig. 23.17).

SAMPLING CONSIDERATIONS

As discussed above, the microbial distribution in foods during a given production period is seldom homogeneous, with microorganisms generally being represented in a nonrandom manner. It is imperative

that the laboratory sample collected for analysis be truly representative of the lot in question.

Quite frequently, an incoming shipment of dried material will contain more than a single manufacturing lot. The sample plan must address this possibility and balance the sampling plan. If the lots are commingled in the shipment, (i.e., it is not a straight shipment, consisting of only one lot) then the Bill of Lading will reflect the composition of the shipment. For instance, if 100 totes of dried milk were received, and 80 were from lot A, 16 from lot B, and 4 from lot C, then representative samples must be taken from each lot.

Compositing of subunits within a lot to make up the laboratory sample is a good way to insure that the analytical unit taken for testing is in fact a true representation of the lot in question.

Since all this is based on statistics, there is no statistical relevance in a sample size of “one” unit. Compositing multiple 25 g units from various containers within a lot to make up the analytical sample is a good way to insure appropriate representation of the lot at large.

When sampling discrete units like bags of dried milk powder, the laboratory sample should always be taken from the top of the bag or unit. Generally, the units are filled and weighed. If the individual unit is over the weight, product is removed and added to a “makeup” box. If the unit is under weight, product is added from the makeup box. In years past, this box

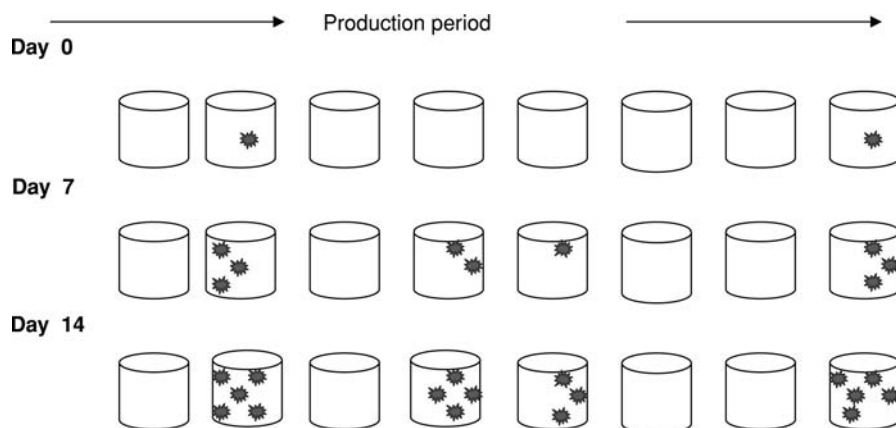


Figure 23.17. Organism growth. (Figure courtesy of Deibel Laboratories, Inc.)

can contain product from multiple lots commingled over various periods of time. It is being replaced by having a coarse fill onto a scale, and then topped off from a product from the same product stream. This change has been seen due to lessons learned in the industry, although it is still very common in bulk butter processors, or other nonautomated packaged products.

Newer packaging equipment utilizes a two-level weighing system: a coarse fill to bring the product near the target weight but not over. This is followed by a fine weigh by using product from the same production lot and ideally the same product stream to bring the container up to the desired weight.

There are several methods for sampling included, “autosampling,” hourly sampling, “beginning, middle, end” sampling, and so on. Samples collected are generally sampled on a shipment basis. Commingled shipments may have different specification from a single lot or “straight” shipments. Various devices are used for sampling fluid products. For dry products, individual companies establish their own sampling plan.

The sampling plan for which lots are selected should be an integral component of the company's overall food-safety program. This should include a strong vendor assurance program with frequent vendor audits, an active daily product release program, historical evaluation of raw material testing data for trending, and identifying the relative microbiological sensitivity of the ingredient in question.

TYPES OF SAMPLES

Raw Material Samples

Raw materials are identified as those individual components used in the production of a finished product. Clearly one company's finished product can be another company's raw material, so these terms only bear weight on the individual company's perspective. Microbiologically sensitive raw materials should be tested on a lot-by-lot basis and used only when cleared by the laboratory. Food ingredients that are relatively micro-insensitive should be evaluated on an audit basis, with testing generally being done once per “x” lots, or on a calendar basis, such as quarterly or annually. Historical data from a supplier should also be used when evaluating the sampling frequency of a food ingredient. It is imperative, however, that standards for testing frequencies be generated by sound scientific data, and not ruled solely by the economics of the situation.

Raw materials can also be tested as a component of a firm's HACCP plan in addition to an overall quality system. HACCP samples are generally taken as “audit samples” of a firm's vendors and are not a component of an ongoing testing program.

Startup Samples

The adage “the product cleans the line” underscores the importance of the startup sample's role in the

detection of bacterial contamination. The very first samples to come off the production line following a line change or an inactive period of time is referred to as the startup sample. In products with high microsensitvity, these samples should be taken and tested without compositing.

In terms of dairy applications, these samples are generally tested for APC, coliforms, *Salmonella*, and *Listeria*.

In-Process Samples

In-process samples are taken to show the movement of entire lots of raw materials through a process, generally when using a pathogen reduction treatment, such as thermo-processing. In-process samples can give detailed information about the adequacy of a process to control the microorganisms present. Additionally, if an organism is being detected in the finished product but cannot be tracked to raw ingredient, “investigative microbiology” using the in-process samples can help determine where in the production process the causative organism has taken up residence. Then targeted sanitation activities can be used to eliminate the contaminant. This type of sampling is used most often when spoilage organisms are causing the decreased shelf life of a product, or when an indicator organism is present in elevated numbers causing rejection of the shipments.

In-process samples should be collected at every possible point in the process where it is feasible to collect a sample without injury to the associate collecting the sample and where the integrity of the product stream can be maintained. Also, these samples should be taken every 1/2–1 hour, or at an interval that yields a high probability of detecting the organism in question.

Line Samples (Finished Products)

Generally, these samples are collected on a time basis throughout the production period of a given lot. The chronologically generated samples are often composited and tested for pathogens such as *Listeria* and *Salmonella*. In the case of a retest for a given lot, the chronological line samples should not be composited but tested individually and with a large analytical unit, such as 375 g, especially for *Salmonella* and *Listeria*.

Finished Product Samples

Many companies test only finished product as this reflects what is being marketed. This testing essentially summarizes the microbiology during the entire processing period, but may consist of line samples taken during the production period. Again, there is no statistical relevance in a sample size of one.

Automatic Sampling

These sampling devices are usually associated with dried products, such as dried milks and whey. This type of sampling offers a superior sampling of the production lot, as long as they are homogenized before testing is performed. They are collected over the entire production period, with a set frequency and sometimes a set collection amount. As each time period is represented as a sediment layer in the collection bag, it is imperative that the bag be adequately shaken prior to taking the firm's library sample and the analytical unit supplied to the laboratory.

Environmental Samples

The most common environmental samples are those taken with a sterile sponge or swabs. These sterile devices are used to sample the surfaces of product contact equipment, other processing equipment, floors control panels, drains, walls, and in some cases skin. Other types of environmental samples do not always involve the use of sponges or swabs, such as standing water in the processing environment, air samples (usually in the form of exposure plates), air filters and accumulated product in a given environmental niche usually associated with processing equipment.

The environmental “zone program,” as seen in current industry trends, is a new approach to organizing a firm's environmental program. The best use of the model is investigative microbiology to track the flow of a pathogen within the plant, while still being able to release product. It should only be used in conjunction with a strong vendor assurance program, microbiological clearance of raw materials before they are used, an active HACCP plan, strong good manufacturing practices, pest control, sanitation checks, and in a test and hold microbiological-release program for sensitive finished products. With all of these systems working in conjunction, the only deficiency is the inability to track the movement of a pathogen

internally in a plant. This deficiency underscores the advantage for the zone program.

Each area of the plant is broken down into one of four zones, generally described simply as Zone 1, Zone 2, and so on.

Zone 1—Product contact surfaces. These are either direct contact surfaces, such as utensils for handling product, conveyors, hoppers, slicers, and so on, as well as those areas of a plant where something can fall into the product stream, such as overheads. All these samples share one main important consideration; they all are directly linked to the product. A “positive pathogen” result has direct product implications. Generally, indicator organisms are tested in Zone 1 samples, such as APC, coliforms, and yeast and molds.

Zone 2—Indirect contact surfaces. These samples are generally on pieces of equipment directly adjacent to the production line, but without direct product implications such as support frames, control panels, equipment housing, utensils not used for direct product contact, and so on. Many times these are physical areas within the production line where one can track the movement of an organism on the hands of a human vector; control panels, handrails, brush handles, and so on. Since there is no direct product implication, pathogens and indicator organisms are generally tested in Zone 2 samples.

Zone 3—The production room. These are areas within the production room, but removed from the production line, and consist of high traffic walkways, drains, wheels on totes or carts, and so on. Since there is no direct product implication, pathogens as well as indicator organisms are generally tested in Zone 3 samples.

Zone 4—Outside the production room. Packaging areas, receiving and shipping rooms, restrooms, cafeterias, and hallways leading up to the production room but from the outside generally make up these samples. They are generally not sampled with as much frequency as the other zones. Like the Zone 2 and 3 samples, since there are no direct product implications, Zone 4 samples are generally tested for pathogens.

The idea of the zone program is to identify each area of interest within the zone, called a site. Each site should be a dedicated object, such as a control panel for operating a hopper, a specific drain, or can be a moving object such as a wheel from a cart carrying milk powder to the packaging machine. In the case of

a nonstationary object, the actual physical item (i.e., front left wheel from bin #1) should be identified on the sample bag in case an investigation occurs later.

Each site should be given an identifier such as 2-1, for Zone 2, site #1. After a detailed assessment of what sites should be chosen, list them on a tracking sheet and identify what sampling frequency best meets the firm's food-safety goals. For instance, company X has 200 Zone 2's and 100 Zone 3's. They produce a dry whey powder and chose to sample each Zone 2 monthly and each Zone 3 quarterly.

Once the zone testing frequency is set, randomize the sites within the zones such that different areas of the plant within a given zone are sampled at each sampling interval. Additionally, randomize the areas within the site being sampled such that different areas within that site are selected. If the site is a large control panel, select all the buttons on the left-hand side during the first round of samples, then once the same site is up for sampling again, select the buttons on the opposite side.

Library Samples

Manufacturers often keep a sample of a production lot for the shelf life of the product plus a short period of time thereafter. If a problem arises in the market place, these samples can provide a reference point. With canned products, such as canned milks and cheeses, it is federally mandated to keep library samples.

Audit Samples

These samples are part of a firm's vendor assurance program and taken during an audit of an ingredient supplier. Audit samples can be environmental samples, in-process samples, finished product samples, and so on. This is another utility of “investigative microbiology,” and should only be used outside of the firm's routine product release program.

HACCP Samples

Most food companies now have HACCP plans that embrace a periodic microbiological profile of the entire operation. This can include pathogen detection, indicator organisms in raw products, environmental samples, and finished products. They are essentially the same as an audit sample but rather written in to HACCP plan with specific frequencies and specifications

Check Samples

These are prepared by an outside laboratory, and inoculated with a known amount of bacteria. Both pathogens, such as *E. coli* O157:H7, *Salmonella*, *Listeria*, and indicator organisms like generic *E. coli*, *S. aureus*, coliforms, and yeast and molds are used. Common check sample suppliers are AOAC, ARI, and Summit Laboratories, and are generally sent out monthly (Summit Laboratories) or quarterly (AOAC and ARI).

The check samples are sent to the laboratory in a blind fashion, along with negative controls of the same product, only not inoculated with the target organism. They are designed to test the laboratory's ability to accurately detect the pathogens in question, and the indicator organisms at the desired levels.

To develop the check sample, the target organism is lyophilized and thoroughly incorporated into a given food matrix such as dried milk, chocolate, and gelatin. The samples are packaged, coded, and sent along with uninoculated product controls to the manufacture's laboratory and to outside laboratories. These establishments can use the samples to validate their procedures as well as providing a valuable training tool for technicians.

Complaint Samples

Occasionally, consumers will call a company about a defect in the product and if that defect is alleged to contain a food-poisoning agent, they are generally referred to the laboratory for testing. These may also have been collected by the sales people in the market place. To avoid the stigma of bias, manufacturers will often use an outside laboratory to run a microbiological profile including common food-poisoning bacteria and indicator organisms.

SAMPLING: THE BEST METHODS OF SAMPLING

"An analysis is only as good as the sampling procedure used to procure the sample. Every effort must be expended to obtain a 'random sample'." Simple "spot sampling" does not yield a representative sample, because there is no statistical validity in a sample size of one sample. For an optimum compositing scheme, it is recommended taking samples on a production basis; for example, one sub-unit every half hour. As a minimum, it is recommended taking a composite sample consisting of startup (or the very first sample

coming off the line at the beginning of production), a middle production sample, and an end sample. The laboratory can then composite these three samples and the analytical unit is taken from these composited samples. Ideally, however, these three samples should be tested individually as the higher the analytical unit used for testing, the greater the opportunity for detecting an organism of concern. Whatever the product and whatever the procedure used to procure the sample, the laboratory sample submitted for testing must be representative of the entire lot of production.

Aseptic Sampling

The importance of aseptic sampling cannot be overstressed. This is a learned technique but often employs common sense, with the overriding principle of strict avoidance of the inadvertent admission of nonsterile matter into the test system. Sterile plastic bags, scoops, tongue depressors, and swabs are commercially available from many supply houses and outside laboratories. Quite often, laboratory personnel perform these duties but in larger organizations, delegated samplers are employed.

In the laboratory, cross contamination can be avoided by using a single container for the media as well as the homogenization or blending steps when considering large analytical units (such as 375 g sample sizes and 4 liters of media). Many laboratories must sterilize 4 liters of preenrichment broth media in autoclaveable polypropylene containers, and then transfer them into blending units along with the composited sample. This clearly adds an additional level of potential contamination to the sample setup process, as multiple sterile devices must be employed per sample. One novel approach to harmonize the media makeup and sample homogenization process is to use a multifunction blending device, such as the Blendo Flask (Summit Lab Supply, Sarasota, FL) (see Fig. 23.18).

Blendo flask offer laboratories a superior sample setup platform, as they use less disposable utensils as well as keeping the potential for cross contamination to a minimum. This process enhances asepsis throughout the sampling and blending steps.

A culture medium is sterilized in the Blendo Flask and composite samples are added, blended, and incubated in the flask. After the incubated culture is transferred into the secondary enrichment, the Blendo flask are heated to destroy the vegetative cells, washed, and are ready for use with new media.



Figure 23.18. Blendo flasks. (Picture courtesy of Summit Lab Supply, Inc.)

Analytical Units

The possibility exists that compositing can dilute a defect to the extent that it is undetectable. However, most pathogen detection assays, qualitative in nature, have been developed to detect very low levels of contamination. It has been demonstrated that one *Salmonella* defective 25 g subunit when cultured with 14 other nondefective 25 g subunits can give a positive result at a 95% confidence level using FDA BAM cultural methods.

Retesting

Many times, there is the desire to retest a sample when specification limits are exceeded or when undesirable microorganisms are detected in a production lot. Sometimes the employment of both larger analytical units (i.e., using a 50 g retest compared to the 25 g original sample size) and multiple samples fail to duplicate the original test result. Two plausible explanations could be either how the sample was taken (sampling), or laboratory error, but there are a num-

ber of other explanations that should be considered. The following examples have been encountered and should aid in understanding the “retest analysis,” or the comparison between original and retest results.

The Statistics Between Original Test and Retest Results

The microbiology behind approving lots of production is governed by statistics. It is the confidence that the analytical unit and the way it was sampled will yield a result that is a good representation of the entire lot of production. The probability of matching original test and retest results is extremely important because economic considerations are involved, but many large companies have adapted the philosophy that you cannot retest away a positive result.

As an example, say, an entire lot of production, representing 3,750 g of product, was sampled by using ten 375 g analyses for *Salmonella* (3,750/375 g = 10 samples). Of these ten samples, only one tests positive for *Salmonella*. Therefore, the level of contamination is one in ten or 10%. Based on standard statistical analysis, if there is a 10% chance of finding the target organism in the original sample, then the ability of finding the organism a second time by retest sampling is (10% × 10%) or (0.10 × 0.10 = 0.01) or one chance in a hundred.

This is a known statistically derived method for determining the probability of finding the same outcome twice. This same formula is used to see the chances of flipping a coin and finding two consecutive “heads”—chance of finding one head is 50%, or one chance in two. The probability of finding a second head is (50% × 50%) or (0.50 × 0.50 = 0.25, which is 25%) or one chance in four.

Similarly, if the level of contamination is 5% the probability of finding a positive the second time is (0.05 × 0.05 = 0.0025, which is 0.25%) or one chance in 400; 1% is one in 0.01% or one chance in 1,000. Table 23.1 presents these considerations.

Summary

The probability of duplicating the original test result is significantly diminished unless the contamination level is relatively high. To obtain significant results, retesting the production lot with some degree of multiplicity is required. *Basically, take many random samples and request a retest as soon as possible.*

The nonrandom distribution of microorganisms in production lots coupled with the production cycle,

Table 23.1. Statistics of Retesting Sample Lots

Level of Contamination	Probability of Original Test and Retest Results that Correlate	
	Percent(%)	Incidence
50%	25	1 in 4
25%	6.25	1 in 16
20%	4	1 in 25
10%	1	1 in 100
5%	0.25	1 in 400
1%	0.01	1 in 1,000

Courtesy of Deibel Laboratories, Inc.

sampling of the product for analysis, the analytical unit analyzed, and the time between original and retest must be taken into consideration when evaluating original and retest results. Retesting must involve an expanded sampling and testing protocol. Sampling and laboratory error are sometimes factors; however, retesting till the desired result is observed is not prudent for food safety.

MICROBIOLOGICAL TESTING IN DAIRY PROCESSING

AEROBIC PLATE COUNTS

The APC is the most widely used and requested indicator test in the dairy industry. This test has many different names such as “heterotrophic plate count” and “standard plate count,” but the industry has somewhat standardized on “APC.” As the name implies, it gives the total aerobic population of a food sample, and is used as an indicator of the total aerobic bacterial population in the sample. It can also be used to identify potential processing issues, sanitation issues, and shelf life determinations on the food matrix.

The test is simple to perform and uses the same pour-plate procedure as outlined in the section “Yeast and Mold,” but it uses “standard methods agar” instead of the potato dextrose agar as in the yeast and mold method. The extrinsic factors of the testing assay can be changed slightly to select for different subpopulations of bacteria, such as incubating the samples at 20–25°C or 50–55°C temperature ranges to for psychrophiles or thermophiles, respectively. Plates can be incubated anaerobically by use of a CO₂-generating system and a sealed jar to select for facultative and strict anaerobic populations in the sample.

Methods for APC

APC FDA BAM, 1998, Revision A, Chapter 3 (1998) (Other methods for APC are available, but the reader is cautioned to only use those tests that have been validated against their specific sample matrix.)

COLIFORMS, FECAL COLIFORMS, AND *E. COLI*

Coliforms are not taxonomically defined and as such do not represent a specific family, genus, or species as in other taxonomic entities. The technical definition is any organism that is capable of fermenting lactose into acid and gas at 35°C in 48 hours. Typically, coliforms are enteric, Gram-negative, and facultatively anaerobic organisms of the family *Enterobacteriaceae*, but not all members of this family are coliforms. They are used as an index of either improper sanitation or improper processing. *E. coli* is abundant in the lower intestinal tract of humans and many animals and excreted in the feces. It was suggested in 1892 by Sheringer (FDA BAM, 1998) that *E. coli* be used as an index organism for recent fecal contamination, which would possibly reflect the presence of pathogenic bacteria, parasites, and viruses. In 1914, the U.S. Public Health Service adopted coliform testing as a screening for sanitary significance (FDA BAM, 1998).

Although the testing procedures for coliforms are easy and straightforward, there was debate as to their ties to fecal contamination due to their isolation from other (nonfecal) sources. Thus a new, nontaxonomical group was introduced as a better indicator of fecal contaminations from the work of various investigators. “Fecal coliforms” are a subset of total coliforms and are capable of producing acid and gas from lactose at elevated temperatures. Testing for fecal coliforms is at 45.5°C versus 35°C for total coliforms, and the group typically includes *E. coli*, *Klebsiella*, and *Enterobacter*. Generally fecal coliform testing is restricted to shellfish and shellfish harvest water, whereas *E. coli* is tested in foods as an indicator of recent fecal contamination.

The scientific community, in the past few years, has begun to question the premise that *E. coli* should be used as a fecal indicator due to free-living *E. coli* found in the processing environment that are not tied to the intestinal tracts of humans or animals. *E. coli* is capable of prolonged survival and growth in environmental niches in a dairy plant, and would, in this case, not be indicative of fecal association.

The most common coliform test is the three-tube MPN assay. It is a statistical-based assay that blends the benefit of a qualitative assay, with results expressed as in a quantitative count. As in most qualitative tests, the analytical unit is significantly larger, giving a higher statistical relevance to the result. In the assay, nine test tubes are separately enriched in the nutrient broth lauryl tryptose broth (LST) at three different dilutions (three test tubes per dilution). The MPN can be used for total coliforms, fecal coliforms, and *E. coli*, in a multistage testing process of presumptive counts and confirm results. The coliform and fecal coliform tests take 48 hours for a presumptive and an additional 48 hours for the confirm result. Both assays start with the same nutrient broth tubes, but the presumptive tubes for fecal coliforms are transferred to *E. coli* broth test tubes and incubated at 45.5°C for 48 hours. Gassing *E. coli* broth tubes represent a positive reaction. Time to result for *E. coli* will take up to 7 days. The testing involves streaking positive fecal coliform tubes onto Levine's Eosin-methylene blue (EMB) plates. Confirmation of the EMB plates is based on a series of tests called IMViC and is outlined in FDA BAM (1998).

There is also a quantitative plate method using Violet Red Bile Agar (VRBA) that is commonly used for coliforms, but the analytical unit is significantly less than in the MPN. The VRBA assay is much faster than the MPN as presumptive results can be given in 24 hours. The sample homogenate (representing a 1:10 dilution) is plated onto a sterile petri plate, and pour-plated with VRBA. The medium is allowed to solidify and capped with more VRBA. This provides a semianaerobic environment forcing coliforms into a fermentation metabolic pathway for the utilization of lactose in the medium. The end products of this reaction are acids, and are detected by the pH indicators in the medium. Coliform colonies will appear as red-purple, with some diffusion of the color into the surrounding medium due to the acidic end products diffusing into the medium.

In the normal MPN, a total of 0.333 g of sample is tested, representing a dilution scheme of $10^{(-1)}$ to $10^{(-3)}$, whereas the analytical unit for the VRBA assay is 0.10 g, a $10^{(-1)}$ dilution only. By utilizing a double strength LST in the MPN assay, the dilution scheme can be increased to $10^{(0)}$ to $10^{(-2)}$ such that a larger analytical unit (3.33 g of sample) is used. Figure 23.19 shows the graphical representation of the MPN testing process.

VRBA can be adopted for *E. coli* by FDA BAM methodologies by the incorporation of 4-methylumbelliferyl-Beta-D-glucuronide (MUG), as all *E. coli* possess the enzyme to cleave this molecule into a fluorescent byproduct. Under ultraviolet light, *E. coli* colonies in VRBA/MUG will fluoresce and should be subjected to further testing to be confirmed as positive. This medium is commercially available.

Methods

Coliforms FDA BAM, 1998, Revision A, Chapter 4 (1998)

Fecal Coliforms FDA BAM, 1998, Revision A, Chapter 4 (1998)

E. coli FDA BAM, 1998, Revision A, Chapter 4 (1998)

(Other methods for Coliforms are available, but the reader is cautioned to only use those tests that have been validated against their specific sample matrix.)

ENTEROBACTERIACEAE

The family *Enterobacteriaceae* includes the lactose-fermenting coliforms as well as other nonlactose fermenting enteric organisms such as *Salmonella* and *Shigella*. They represent a larger group of potential pathogens and indicator organisms than tests for coliforms alone. The dairy industry has begun to use this testing as index organisms for sanitation and indicator organisms of concern as it also tests indirectly for *Salmonella*. The largest use of this assay is in environmental sponge or swab testing as food contact surfaces can be tested indirectly for *Salmonella* without direct product implications for the pathogen.

The testing takes advantage of the VRBA agar method but with the supplement of glucose, as all *Enterobacteriaceae* can ferment this carbohydrate.

Methods

Enterobacteriaceae, Compendium for the Microbiological Examination of Foods, 4th edition (Downes and Kieth, 2001)

(Other methods for *Enterobacteriaceae* are available, but the reader is cautioned to only use those tests that have validated against their specific sample matrix.)

YEAST AND MOLDS

Yeasts and molds (YMs) are common spoilage organisms in the dairy industry. Molds are used to determine "the inadvertent admission of moisture into the food manufacturing process." YMs are also used for determinations for when the shelf life endpoint

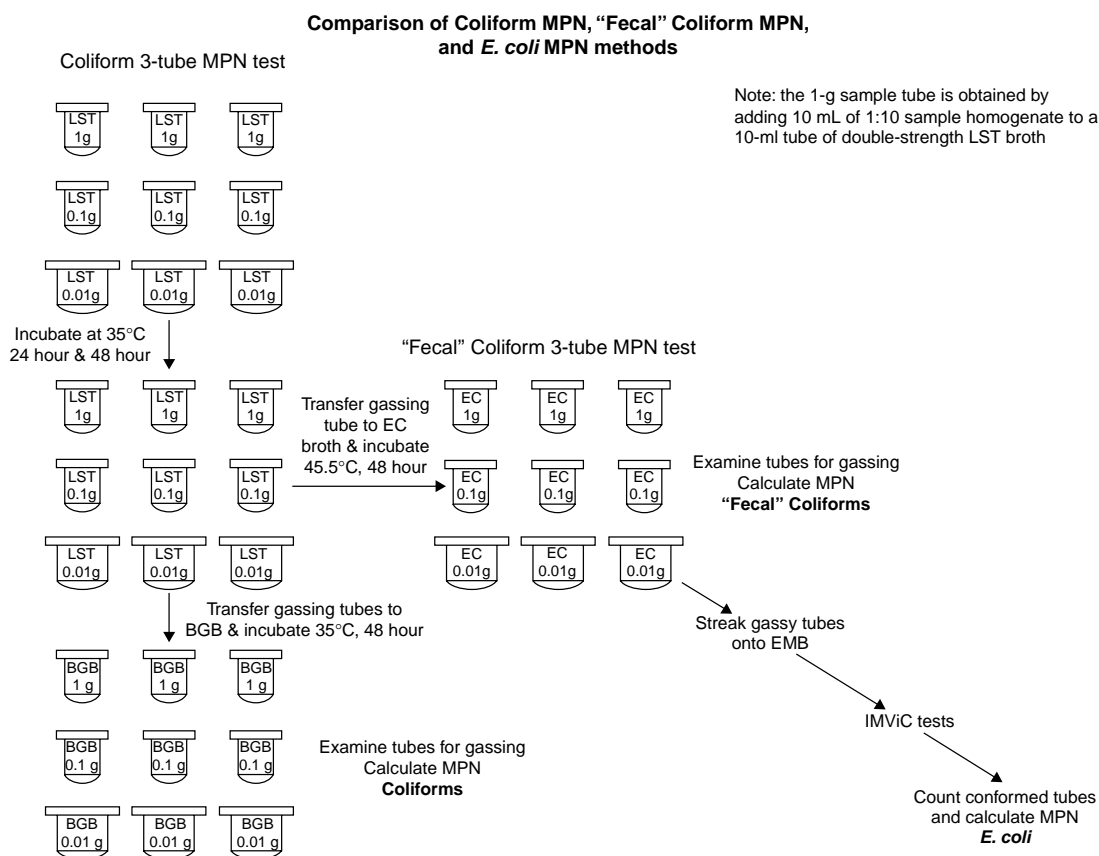


Figure 23.19. Diagrammatic representation of the MPN assay for coliforms, fecal coliforms, and *E. coli*. (Picture courtesy of Deibel Laboratories, Inc.)

of a product as been surpassed. The dairy industry does not have to be concerned with some of the more fastidious fungal organisms, such as halophiles (salt loving) or osmophiles (sugar loving). Only rarely are halophiles encountered in cheese brines. As such, the basic testing methodologies, as outlined in FDA BAM using potato dextrose agar (PDA), are sufficient for enumerations of YMs in the dairy industry.

Molds are strict aerobes and do not grow in anaerobic environments. However, the common FDA BAM procedure used by testing laboratories is a pour-plate method, providing a decreased aerobic environment in the bottom of the petri plate. This may result in false low enumeration counts. Other spread-plate methods are available such as one using Rose Bengal agar, which is an excellent recovery medium, but is not used as frequently as the PDA pour-plate method. Rose Bengal slightly inhibits mold growth and is

ideal for enumerating larger numbers of molds, as the colonies would not spread across the plate, obscuring other mold colonies from detection.

Yeasts are mostly facultative in regards to oxygen requirements and capable of growth under aerobic or anaerobic conditions. They are generally indifferent to the pour-plate versus spread-plate dilemma as seen in mold enumerations.

Yeast and Molds: Issues in Culturing

The main issue in culturing yeast and molds is the time to result. The three main factors in choosing a test method are cost, time-to-result, and data quality with generally only two of the three being possible. The methods currently available for YM enumerations are reliable and reproducible. But the typical

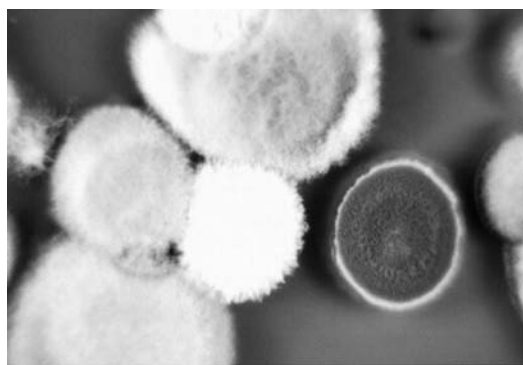


Figure 23.20. Overcrowding of molds on PDA. Picture of common PDA plate with mold colonies growing over each other. (Picture courtesy of Deibel Laboratories, Inc.)

enumerations take up to 7 days, placing an increased burden on a dairy plant's supply chain, distribution, and warehousing costs. Rapid methods are fast and reproducible but the data quality is an issue, as most do not correlate well in comparison to standard FDA BAM cultural methods.

Procedure: FDA BAM Enumeration Counts

The BAM protocol is to incubate the PDA plates for 5 days at 25°C. If no molds are detected, the plates are to be incubated for another 48 hours (see Fig. 23.20).

Rapid Fungal Methods

Yeasts have shorter generation time than molds, so they behave more like bacteria. Yeast counts generally take 2–3 days versus the 5–7 days with molds. As of this date, no comprehensive evaluations have been performed on rapid YMs kits. Most studies are done by the food manufacturer or by a private laboratory; and in each case, the studies are held proprietary and are generally only specific for a narrow scope of food matrices. As a generality, most studies do not show good enough correlation between cultural methods and the rapid method, with the added drawback that the rapid methods robustness on a variety of food matrices has not been adequately explored.

Methods

YM by Spread Plate FDA BAM Chapter 18 (1998)

YM by Pour Plate FDA BAM Chapter 18 (1998)

(Other methods for YM are available, but the reader is cautioned to only use those assays that have been validated against their specific sample matrix.)

MYCOTOXINS IN ANIMAL FEEDS

Certain molds can produce a secondary metabolite (i.e., one not required by the mold) that is highly toxic. This group of chemically diverse compounds is referred to as mycotoxins and the primary toxin of interest to the dairy industry is aflatoxin B₁ produced by certain, not all, strains of *Aspergillus flavus*. Cows that ingest moldy hay or grain may consume this toxin and convert it to a hydrolyzed form called aflatoxin M₁ which can be secreted in the milk. The latter is still quite toxic to humans and the FDA has established an actionable level of 0.5 µg/kg for milk products.

Qualitative and quantitative assays for aflatoxin M₁ are commercially available and an HPLC procedure received final action by AOAC (2006). The HPLC methods require the use of expensive analytical equipment are tedious and require a well-trained analyst. ELISA testing platforms, such as “lateral flow devices,” offer a faster time-to-result, are more cost-effective, and can be performed using relatively little training. However, these testing platforms might not have FDA or AOAC approval for use on the type of food matrix in question.

Methods

Aflatoxin by Thin Layer Chromatography AOAC 974.17 (2006)

(Other methods for mycotoxin detection are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

PATHOGENIC BACTERIA IN DAIRY FOODS

The main pathogens of concern in the dairy industry are *Salmonella* and *Listeria monocytogenes*. Two other organisms that have also proven problematic are *E. coli* and *S. aureus*, although they are less frequently involved in outbreaks or recalls. *S. aureus* is notable for outbreaks in milk resulting in enterotoxin production and subsequent food-poisoning outbreaks. The dairy industry is also at increased susceptibility to these organisms due to their predominately wet processing environments (see Fig. 23.21).

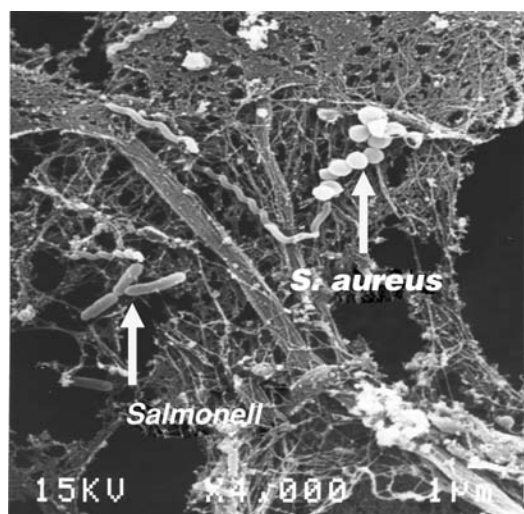


Figure 23.21. Micrograph of *Salmonella* and *S. aureus* cells. (Micrograph courtesy of Dr Peter Cooke.)

QUALITATIVE ASSAYS FOR PATHOGENIC BACTERIA—*SALMONELLA* AND *LISTERIA*

Assays for detecting pathogenic bacteria in dairy foods generally follow the same general scheme, and almost always involve qualitative testing, with Boolean results expressed as “Positive” or “Negative.” One exception to this is when detecting *S. aureus* where quantitation may be necessary or detection of the enterotoxin. Techniques involve resuscitating the organisms in nonselective media that aid in the recovery of sublethally injured cells. The cultures are transferred into increasingly selective media, ending in the target organism being represented at high enough levels to detect either by physiological and/or serological procedures.

The concept of resuscitating the bacteria is of key importance as pathogenic organisms found in food products may have been damaged by the manufacturing process. Thermo-processing, the intrinsic factors of the food matrix (such as the foods, a_w , preservative systems, pH, and salt content), or by extrinsic factors such as the storage conditions or if a modified atmospheric packaging (MAP) was employed.

SALMONELLA

Salmonella (Deibel et al., 2005) are a major concern in the dairy industry. They have led to more recalls and outbreaks than any other food-borne organ-

ism. Unlike *Listeria* with a relatively high mortality in susceptible populations, it rarely causes fatalities as a food-borne agent in most developed countries. However, where water sources are contaminated by human sewage, such as in developing countries or those areas suffering from a catastrophic event, *S. typhi* and *S. paratyphi* can cause severe illness, resulting in higher mortality rates than those food-borne outbreaks in the North American, Europe, and other developed countries.

Salmonella have been implicated in food outbreaks with nearly every dairy product, including raw milk, cheeses, ice creams, ice cream premixes, milk powder, whey powder, fermented products like yogurt and cottage cheese, milk chocolates, and even as a contaminant in starter cultures, both bacterial and fungal. Species of this genus can survive very adverse conditions such as high acid concentrations, high salt concentrations, very dry environments, and in environmental niches within the production facility.

Organism Characteristics

Salmonella are Gram-negative nonsporeforming bacilli and facultative in their oxygen requirements. Members of this genus belong to the *Enterobacteriaceae* Family and all are considered pathogenic by FDA, but not all are pathogenic for humans. Several host-specific strains, primarily chicken and horse, have not been involved in a human incidence of Salmonellosis.

Salmonella can be found in almost any food. Some of the foods implicated in past outbreaks include cheese, raw milk, chocolate, soy flour, egg-containing dishes, peanut butter, poultry, pork, and ice cream. The infective dose ranges from 1 to 100,000 cells and is influenced by the food product, serotype, and individual susceptibility. The hosts that *Salmonella* are primarily associated with include birds, reptiles, insects, and animals including humans.

Taxonomy

Currently, there are two *Salmonella* species (*typhi* and *typhimurium*) with a total of more than 4,000 serotypes. However, most names are just expressed as the genus, *Salmonella*, and the serotype, such as *S. newport*, *S. enteritidis*, and *S. cubana*. All serotypes are considered to be pathogenic in humans. Most food-borne species cause a gastroenteritis infection

with onset generally 12–36 hours after consumption of the contaminated food product. Symptoms include abdominal pain, diarrhea, nausea, vomiting, mild fever, and chills. Duration is generally 2 to 5 days. There are two other types of Salmonellosis (both classified as enteric fevers): Typhoid fever (*S. typhi*) and paratyphoid fever (i.e., *S. paratyphi* A and *S. paratyphi* B). While these two types can be spread by the food-borne route or via a human carrier, they are more commonly associated with contaminated water. Typhoid fever is the most severe of all the diseases caused by *Salmonella* but fortunately it is not as prevalent today as it once was. This is largely due to improved water/sewage treatment facilities. Today, typhoid fever is occasionally still a problem in underdeveloped countries especially in situations where there is crowding of people and a breakdown in sanitation (i.e., war and natural disasters).

Onset is generally 7–21 days after exposure, while symptoms commonly include rose-colored spots on the abdomen, high fever, nosebleed, headache, and loss of appetite. The organism invades the lymphatic system and subsequently invades the entire body, producing an overall generalized (systemic) infection. *S. typhi* can be isolated from the blood and bone marrow and it has the ability to attack many organs (i.e., gallbladder and brain). Once infected, a human can harbor the organism indefinitely. Carriers can excrete the organism intermittently either fecally or less commonly via the urinary tract. The mortality rate of typhoid fever is approximately 10% with a carrier rate of an estimated 2%. Carriers represent a threat to the general population especially when allowed to become food handlers, as in the case of infamous “Typhoid Mary.” The paratyphoid fevers are similar to *S. typhi* infections but tend to be considerably milder. A sudden onset accompanied with chills is typical of the paratyphoid infections. The incubation period is shorter and the organisms are generally not isolated from the blood. Both *S. typhi* and the paratyphoid species are strict parasites of humans (i.e., host-adapted). If they multiply outside of the human host, it is believed to be insignificant in the spread of these diseases.

Because of mutation and genetic exchange in the microbial world, there are an increasing number of *Salmonella* strains that do not conform to the standard criteria. For example, typical *Salmonella* do not ferment the carbohydrate lactose, and do produce hydrogen sulfide (H_2S); however, an isolate from a food sample may be lactose positive or H_2S neg-

ative. These “atypical” variants are still recovered on the plating and tubed media that are used for *Salmonella* isolation and identification by standard FDA BAM and USDA FSIS cultural procedures. The microbiologist must become familiar with how “typical” and “atypical” *Salmonella* appear on these media and pick all “presumptive positives” for further biochemical work-up. Genetic and immunological assays (PCR and ELISA, respectively) make up the backbone of common “rapid” testing assays, and can also detect the variant *Salmonella* species.

Sources

Most animals are capable of being reservoir hosts for *Salmonella*, including most mammals, birds, reptiles, and also insects such as moths, ants, and cockroaches. The host range is practically unlimited.

Foods Implicated in Illness

Most raw or under-cooked foods have the potential of having *Salmonella*, but cross-contaminated foods of animal origin also play an integral role in *Salmonella* outbreaks. Egg-containing dishes (especially those containing raw eggs), poultry sources, and milk-based products play the largest role in *Salmonella* outbreaks.

Control and Prevention

Proper prevention of cross-contamination between raw and ready-to-eat foods and thorough cooking (preferably with moist heat) provide the two best mechanisms for control of *Salmonella*. The organism dies at ca. 48.9°C if properly hydrated. There is typically no growth below ca. 7.2°C. *Salmonella* are killed in a normal milk pasteurization procedure; however, the species have shown remarkable abilities to survive in very dry environments, especially when these dry product matrices are subjected to thermal processing.

Methods for the Detection of *Salmonella* in Dairy Products

The isolation protocol for *Salmonella* varies depending on the food type (cheese, chocolate, whey powder, meat, etc.) and the method to be used (FDA-BAM, FSIS, one of the rapid methodologies). All cultural methodologies involve some sort of a preenrichment

which helps to revive injured cells, dilute out possible toxic components in the food itself, and provide a growth medium for the cells. The typical preenrichment for dairy products is lactose broth, nonfat dry milk is used for all confectionary products, including milk chocolates.

Once the cells have had a chance to recover and multiply, they are subcultured to selective enrichment broths (i.e., Selenite Cysteine, Tetrathionate, Rappaport Vassiliadis/R10), which helps to inhibit the growth of “competitors” and allow *Salmonella* to further multiply. Cultures are then streaked onto selective/differential plating media. The plates help to select for *Salmonella* by incorporating ingredients (i.e., Brilliant Green, Bile Salts) that will discourage the growth of many competitors but still allow *Salmonella* to grow. The plating media also incorporate differential agents that allow the distinguishing of *Salmonella* from competitors, with most differential ingredients generally composed of amino acids or sugars. Organisms able to utilize these media additives will produce alkaline or acidic byproducts that can be detected by a color change evidenced by the pH indicator present in the medium.

When looking at the various media used for *Salmonella*, it is important to remember that there are over 4,000 serotypes, and it should be stressed that there is a considerable amount of variation within the genus. Because of these considerations, there are multiple media used for the secondary enrichments and for plating. It is critical to achieve isolated colonies on the plating media or a positive *Salmonella* could be overlooked. Plates should always be “streaked for isolation.” Unfortunately, this sometimes delays results. However, if colonies are not isolated and a “mixed” or contaminated culture is isolated, the subsequent biochemical and/or serological reactions can be obscured and the sample could be reported falsely as being negative.

Isolated colonies that are suspect for *Salmonella* are inoculated into biochemical media (LIA and TSI tubes) and a broth culture (TSB-YE) for antigenic characterization. Isolates that exhibit suspect biochemical reactions in either the LIA or TSI are subjected to “poly-H” and tested in order to determine if a *Salmonella* species is present. All the *Salmonella* H-antigens are typical only to *Salmonella* and to no other organism. In this way, the definitive *Salmonella* confirmation test is the poly-H, which contains a mixture of known antibodies to most but not all *Salmonella* flagella (H) antigens. If a

complete species determination is desired, the exact antigenic make up of their somatic or “O” antigens and their flagella, or “H” antigens are determined. This can be useful to pinpoint the source of the contamination (i.e., raw ingredient from a supplier or environmental contamination, etc.) Therefore, all positive isolates should be saved until it is decided if speciation is necessary.

***Salmonella* Confirmation Poly-H Method**

This is the definitive assay for *Salmonella*, but must be carried out in an aseptic manner using a pure culture. A cross-contamination on this test can result in a “false-negative” result.

1. Add 3 mL of formalized saline to the 3 mL TSB-YE culture and gently mix.
2. In a 10 × 75 mm test tube, add 0.3 mL of formalized culture to 0.3 mL of poly-H antisera (commercially available) and flame off the lip of the tube.
3. Place rack in the 50°C water bath for 1 hour.
4. Observe for cell agglutination on the bottom of the tube.

Positive reaction: Agglutination—the solution clears and a precipitate forms.

Negative reaction: No agglutination—the solution remains uniformly cloudy (see Figs. 23.22 and 23.23).

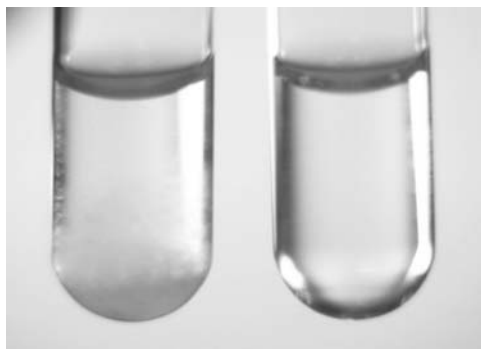


Figure 23.22. Poly-H Reactions for *Salmonella*. Positive Poly-H agglutination reaction (tube on left); Negative Poly-H reaction (tube on right). (Picture courtesy of Deibel Laboratories, Inc.)

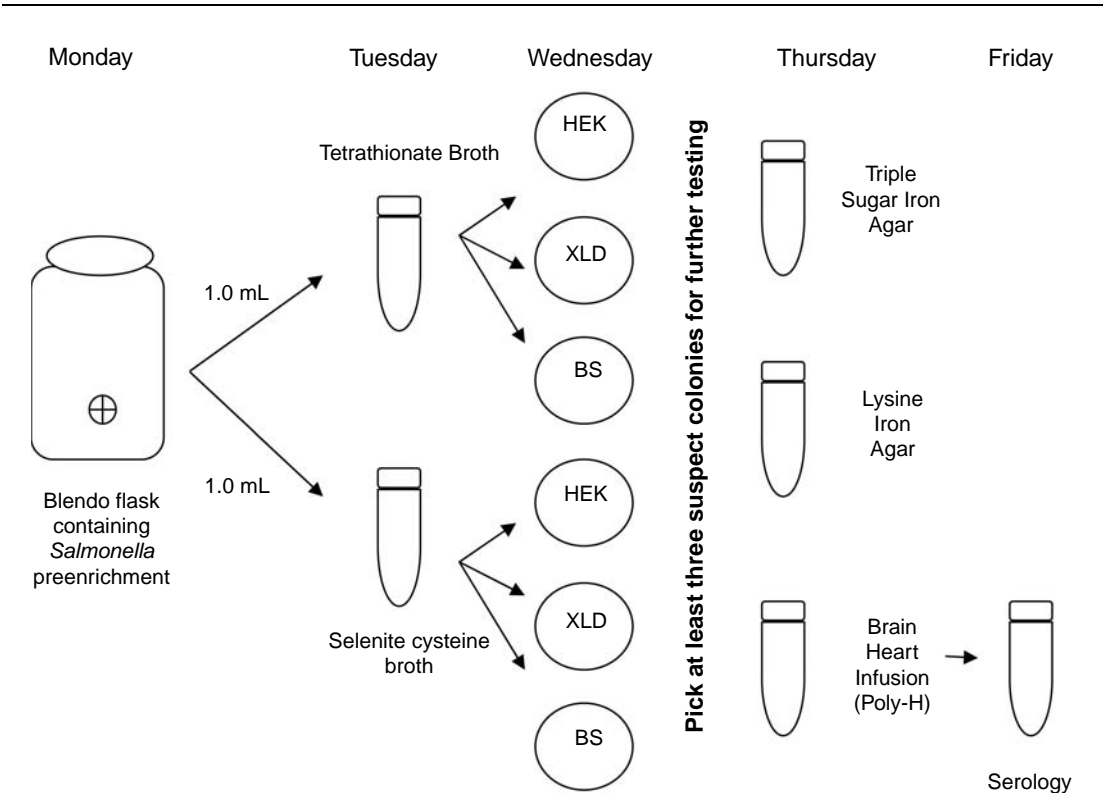


Figure 23.23. Diagram of a FDA BAM *Salmonella* assay for the isolation of *Salmonella* in food samples. (Diagram courtesy of Deibel Laboratories, Inc.)

Methods for *Salmonella* Detection
Salmonella FDA BAM, Revision A, 1998, Chapter 5 (1998)
Salmonella by USDA FSIS Microbiology Laboratory Guidebook Chapter 4, Revision 3 (USDA)
(Other methods for *Salmonella* are available, but the reader is cautioned to only use those methods that have been validated against their specific sample matrix. A more complete list of rapid methods is available through AOAC and FDA BAM, Appendix 1 to *Salmonella* Method, Chapter 5.)

LISTERIA

Listeria (Deibel et al., 2005), specifically *L. monocytogenes*, was only considered a concern for the livestock industry until the mid-1980s. It has since surged into the consciousness of most Americans,

becoming almost a household name to the average consumer, behind only *E. coli* and *Salmonella*. The first major food-borne outbreak with a dairy product involving listeriosis, or the infection caused by *L. monocytogenes*, involved the Jalisco Mexican style “soft-cheese” outbreak of 1985 in California. The facility was located in Los Angeles, and was under pressure to produce more cheese than their pasteurization tanks had the capacity to hold. Raw, unpasteurized milk was used, resulting in 142 cases and 48 deaths. Since this time, food-borne outbreaks involving *Listeria* have been splashed across newspapers and magazines mainly due to this organism’s almost insidious predilection for immune compromised individuals, especially pregnant women and young children. Most outbreaks have occurred in ready-to-eat meat products and in fresh produce, but a fair amount has occurred in dairy products, primarily

cheeses. Two of the only dairy products that have not seen an *L. monocytogenes* outbreak up to this point has been in milk chocolate products and ice cream.

Listeria are ubiquitous in nature. They are associated with cattle and sheep as well as other farm animals. They are also found in soil, plants, decaying vegetation, sewage, surface waters, silage, and animal feces. There have been numerous outbreaks where the source of contamination for vegetables has been traced back to fertilizer from infected farm animals. The organism may be found in almost any food. Because *Listeria* is so widely distributed in nature, there are many possible transmission vectors. Foods that can be traced back to an animal source are more suspect.

This organism is difficult to control not only because of its ubiquitous nature but also because of certain physiological features including its ability to survive in adverse environmental niches indefinitely. Of particular interest to the dairy industry is this organism's psychrophilic growth range, with identifiable growth seen almost down to freezing. These organisms, however, are very sensitive to higher temperatures, and will be rendered nonviable through the normal milk pasteurization process (i.e., 79.4°C for 15 seconds). *Listeria* lose their ability to produce flagella at 35°C, so from a research perspective, it seems likely that this is not necessary to promulgate the infection in the mammalian hosts cells.

Of concern to most dairy plants is that the organism seems to establish itself in wet, cold-processing areas. *Listeria* are also very well suited for prolonged survivability in austere environments, including growth on stainless steel equipment and their ability to develop slight resistances to quaternary ammonium sulfates as well as certain other sanitizing chemicals after prolonged use.

It appears that this organism has a disproportionate mortality rate when compared to a healthy adult, which would display only a mild or unremarkable fever, if any symptoms are seen at all. This is in contrast to the nearly 30% mortality rate as seen in sensitive individuals. The main at-risk populations are pregnant women, young children, the elderly, or other immune-compromised persons, such as those with AIDS, cancer, and diabetes.

On the basis of taxonomical identifications there are six *Listeria* species, *innocua*, *welshmerii*, *sellegerii*, *grayii*, *ivanovii*, and *monocytogenes*, with

the latter being classified as the only organism seen as a food-borne pathogen. However, *L. ivanovii* is currently also being classified as a human and animal pathogen, although it has not been involved in any food-borne outbreaks at this time.

Of particular interest to the dairy industry are the differences in government reactions (oversight) to *Listeria*. In the European Union and Canada, there are quantitative specifications for *L. monocytogenes* (LM), anywhere from 100 LM/g to 10 LM/g in foods not intended for the susceptible population. By contrast, there is a zero tolerance for *Listeria* in all ready-to-eat foods and dairy products. In relative terms, this genus does not compete well with the normal background flora seen in food products, where bacterial growth is feasible. At the species level, LM, as well as most other *Listeria* sp., will be outgrown by *L. innocua* (LI). This is by far the most prevalent *Listeria* species encountered by testing laboratories. Some studies show that LI will outgrow all the other *Listeria* species and be represented in the food matrix at a ratio of 5:1.

Organism Characteristics

Listeria are Gram-positive, flagellated nonspore-forming, and cocci-bacilli (very short rods, almost like a pinto bean) and are considered facultative anaerobes in their oxygen requirements, meaning they prefer an anaerobic environment, but are very capable of growth in aerobic conditions. They do, however, prefer oxygen levels slightly lower than atmospheric conditions, so they are more correctly referred to as microaerophilic-facultative anaerobes. This microaerophilic characteristic of the genus plays a very important role in the certain biochemical testing (see Fig. 23.24, Motility Umbrella below), when laboratories are asked to identify *Listeria* in a food sample when using standard cultural FDA or USDA methodologies, or when confirming a presumptive positive result from common ELISA or PCR "rapid" methods.

Listeriosis, or the infection caused by *Listeria*, initially can result in nausea, vomiting, abdominal pain, and fever, but symptoms in the individuals can be extremely variable. A bacteremia can eventually develop and progress to life-threatening conditions such as meningitis and encephalitis. Pregnant females may develop flu-like symptoms that are relatively mild. The risk to the fetus can be much more severe causing



Figure 23.24. *Listeria* Gram stain. Gram stain of *Listeria* culture. (Picture courtesy of Deibel Laboratories, Inc.)

spontaneous abortions or stillbirths. The mother may also pass this organism onto the neonate as it passes through the birth canal. In this case, the infant usually develops encephalitis and dies soon after being born. The death rate can be high in infants, the elderly, and the immunocompromised with a case fatality rate estimated between 25–30%. The infectious dose is currently unknown but is believed to be as few as 100 cells. The incubation period is typically several days to as long as several weeks. However, when heavily contaminated food is ingested, the incubation period may be as short as a day. The incidence is difficult to determine because of the long incubation period in many cases. This makes it difficult to pinpoint a source when illness does not occur until several weeks after ingestion of the contaminated product.

Taxonomy

L. monocytogenes is widely regarded as the only one of the six *Listeria* species that is pathogenic to both humans and animals. However, *L. ivanovii* is currently also being classified as a human and animal pathogen, although it has not been involved in any food-borne outbreaks at this time. A closely related species, *L. innocua*, is common in foods but is nonpathogenic. Strains of *L. monocytogenes* recovered from foods may exhibit a variety of phenotypic characteristics; not all of these strains will fit the classical definition of *L. monocytogenes* (e.g., that outlined in

Bergey's Manual of Systematic Bacteriology). In particular, food-derived *L. monocytogenes* may exhibit a range of hemolytic activity and weakly hemolytic or nonhemolytic isolates may be confused with *L. innocua*.

Culturing Procedures for *Listeria*

Listeria assays in most rapid and nonrapid procedures follow a slightly different scheme than those for other pathogens, in that they do not use a nonselective medium as a preenrichment. Unlike *Salmonella*, *Listeria* are cultured primarily in selective media such as *Listeria* Enrichment Broth (LEB), University of Vermont Medium (UVM), or Demi-Fraser broth. Demi-Fraser is also called Half-Fraser, because it differs from Fraser broth only in that it uses half the concentration of certain key antimicrobial agents. All of aforementioned *Listeria* media use a fairly high amount of selective ingredients to limit the growth of Gram-negative bacteria, and competitive background Gram-positive microflora such as streptococci, bacilli, and lactic acid bacteria.

Regardless of the preenrichment, all food matrices require a 48 hours enrichment scheme, some methods just maintain one media for the entire 48 hours, like FDA BAM, while most require a transfer into a more selective media after 24 hours, such as in most ELISA assays, PCR assays, and USDA cultural methods. Some companies that market a 24 hour *Listeria* assay (shortening the time-to-result seen in other assay almost in half) but these procedures seem to be specific to individual food matrices or environmental sponges/swab samples, and not recommended across the board for all food types.

Methods for the Isolation of *Listeria* in Dairy Products

Both the FDA and the USDA procedures employ a selective enrichment broth as the initial step in the recovery of *Listeria* species. Both these procedures yield a qualitative result and neither procedure incorporates a step for the resuscitation of injured or stressed cells. The sample is enriched in one of the several (depending on the methodology) broths that contain selective antibiotics. The revised FDA procedure incorporates a 4 hours nonselective enrichment



Figure 23.25. Positive reaction on motility medium; “Umbrella” growth of *Listeria* spp. (Courtesy of Deibel Laboratories, Inc.)

at 30°C before the addition of three different antibiotics (cycloheximide, naladixic acid, and acriflavin). The addition of pyruvate also aids in the recovery of sublethally injured cells. After incubation, samples are streaked to selective/differential plating media (MOX and/or PALCAM) and may additionally be transferred to secondary enrichment broths (such as Fraser broth incorporated in the USDA procedure). The MOX, PALCAM, and Fraser broth all incorporate esculin as the differential ingredient. All *Listeria* are able to hydrolyze esculin and produce a coumarin glycoside as the end product. The glycoside reacts with ferric ions in the media (present in the form of ferric ammonium citrate) and produces a dark brown or black precipitate. Presumptive colonies are subjected to physiological tests such as a Gram stain (Fig. 23.24), the presence of an “umbrella” (Fig. 23.25), and “tumbling” motility as seen under a wet mount slide and 100× objective. All testing done to observe the motility of *Listeria* must be characterized at 25°C, as flagella production does not occur above 30°C.

Industry has various methods employed for routine surveillance of *Listeria*. Some companies will react to any “MOX Positive” result or any suspect colony on MOX that shows characteristic *Listeria* colonies. Others react to LM, so a typical colony on MOX is struck onto MOX overlaid with horse blood agar. Hemolytic colonies are suspect LM after 22–24 hours incubation at 35°C, and isolates are subjected to further testing to confirm LM. Lastly, some

companies will react to any confirmed *Listeria*, so suspect colonies from MOX are subjected to catalase testing, motility medium to look for “umbrella” formation, and a wet mount to observe tumbling motility under microscopic evaluation.

The two widely used cultural assays, by standard FDA BAM or USDA procedures, are summarized in Fig. 23.26.

If an exact species is required, additional testing is performed such as sugar fermentation reactions (rhamnose, xylose) and a hemolysis assay known as the CAMP test (Figs. 23.27, 23.28, and 23.29, respectively).

Motility medium is used to show the microaerophilic growth characteristics of *Listeria* sp. The analyst will stab a motility medium test tube from a well-isolated colony, and observe the tube for an “umbrella” type of growth (Fig. 23.25), after incubation at 25°C for 1–7 days.

The umbrella may not be pronounced after 24 hours of incubation. Examine hanging drop preparations of the TSB-YE culture for “tumbling” motility as follows: prepare a slide by spreading Vaseline at the edges of a concave microscope slide. Place a drop of the TSB-YE culture in the middle of a coverslip and invert the microscope slide on top of it. Examine under oil using the 100× objective of the microscope. *Listeria* cultures will be short rods that exhibit an end over end (“tumbling”) motility.

Because of phenotypic variants, isolates may not always fit into a certain species designation, but the typical reactions are summarized in Table 23.2. For example, the typically hemolytic LM may be weakly hemolytic or nonhemolytic. Sugar fermentation patterns are also variable. A species that typically ferments the sugar rhamnose (i.e., *L. monocytogenes*) may be rhamnose negative. When uncharacteristic reactions are observed the analyst should check the isolate for purity. Observing the umbrella reaction or examining a wet mount preparation under the microscope will usually suffice. If the culture is mixed, it has to be reisolated before species characterization can be performed. If the culture is not mixed, it may be helpful to subject the isolate to one of the rapid identification systems commercially available (see FDA Appendix 1a and 1b for test kits for *Listeria* species identifications (FDA, 1998).

Using the TSB-YE culture, transfer a drop of all cultures to separate tubes of Purple broth (9 mL) supplemented with 1 mL of a 10% filtered-sterilized sugar

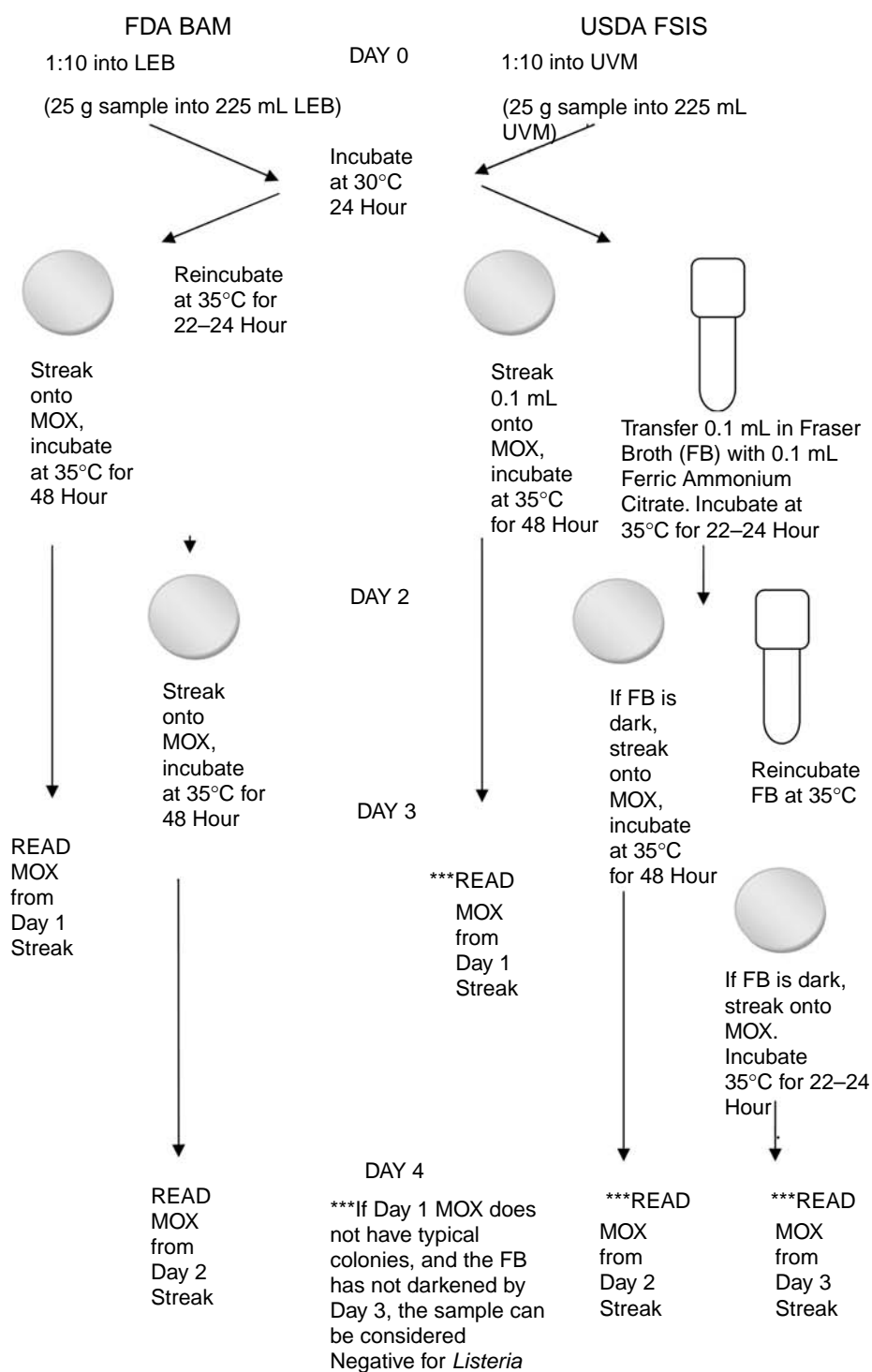


Figure 23.26. A diagrammatic comparison of FDA and USDA procedures for the isolation of *Listeria*. (Diagram courtesy of Deibel Laboratories, Inc.)

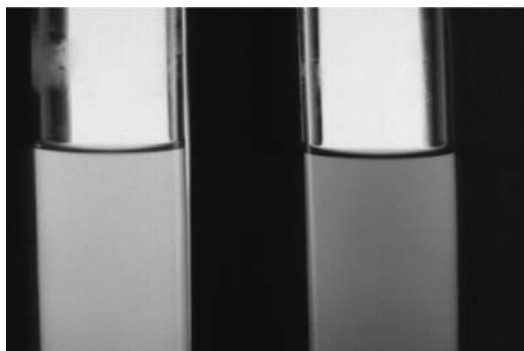


Figure 23.27. Positive sugar reaction in BCP broth after incubation. Left: positive BCP reaction. Right: negative BCP reaction. (Picture courtesy of Deibel Laboratories, Inc.)

solution of rhamnose and xylose. Incubate the sugars at 35°C for 18–24 hours.

Additionally, each isolate is struck to check for hemolytic ability on a CAMP blood plate. These preprepared plates contain 5% sheep's blood and are commercially available through Remel or Difco. A B-lysin producing strain of *S. aureus* and *R. equi* (available through AOAC, or Summit Laboratory Supply) are streaked in parallel onto a blood plate. The test strains of *Listeria* are streaked at right angles to the *S. aureus* and *R. equi*, such that the isolates are struck

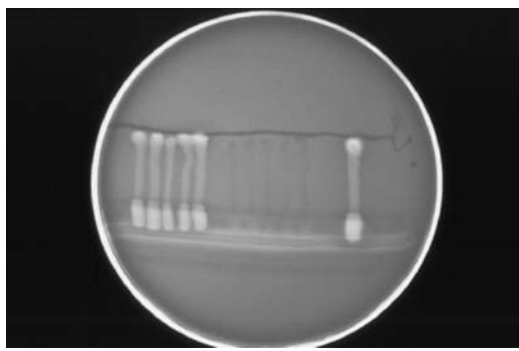


Figure 23.28. Photograph of a CAMP plate containing *L. monocytogenes* (LM) and non-LM isolates. *S. aureus* is the lower horizontal streak. *R. equi* is the upper horizontal streak. The first five isolates (reading from left to right) were identified as LM and the next five isolates gave a negative reaction. The far-right isolate is a control culture of LM. (Picture courtesy of Deibel Laboratories, Inc.)

like rungs on a ladder, and either side of the ladder is the *S. aureus* and *R. equi* streaks (Figs. 23.28 and 23.29).

These two organisms produce substances that may enhance the hemolysis of certain *Listeria* species. Streak the test strains up to the *S. aureus* and *R. equi* but do not allow them to touch. In addition, make an "X" in the center of the test strain to detect

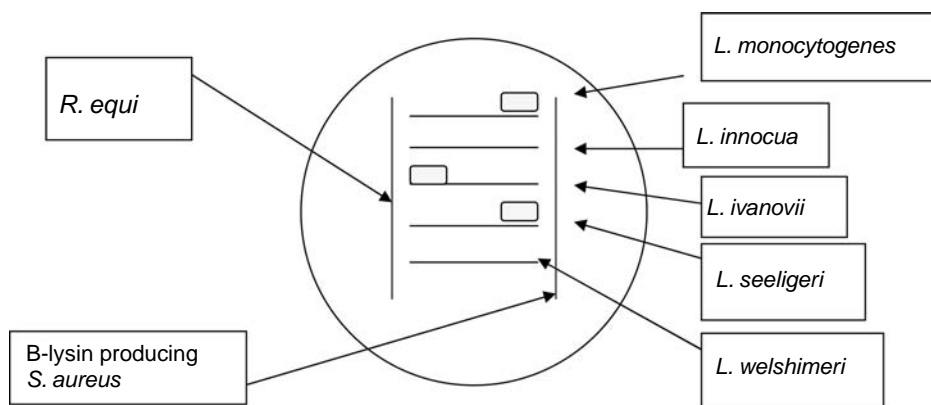


Figure 23.29. Diagrammatic representation of *Listeria* species on CAMP test. *L. monocytogenes* and *L. seeligeri* are hemolytic (as evidenced by a clearing) with *S. aureus*. Some strains of *L. monocytogenes* are additionally weakly hemolytic with the *R. equi*. It is possible to distinguish between the two species by looking at the sugar fermentation reactions (see Table 23.1). (Illustration courtesy of Deibel Laboratories, Inc.)

Table 23.2. Classic *Listeria* Reactions

<i>Listeria</i> Species	Rhamnose Reaction	Xylose Reaction	Hemolysis with <i>S. aureus</i> (CAMP Test)	Hemolysis with <i>R. equi</i> (CAMP Test)
<i>L. monocytogenes</i>	+	–	+	–or ±
<i>L. ivanovii</i>	–	+	–	+
<i>L. innocua</i>	V	–	–	–
<i>L. welshimeri</i>	V	+	–	–
<i>L. seeligeri</i>	–B	+	+	–

V, Variable (+/–).
Note: Some strains of *L. monocytogenes* will also react with the *R. equi* in addition to the B-lysin *S. aureus* (– or ±).
(Courtesy of Deibel Laboratories, Inc.)

any weakly hemolytic organisms. *Listeria* produces a Listeriolysin (hemolysin) that is somewhat oxygen sensitive. The hemolytic reaction is therefore more pronounced in the depths of the agar where there is less oxygen. Incubate the blood plate at 35°C for 18–24 hours.

Like *Salmonella*, *Listeria* have both O (somatic) and H (flagellar antigens; however, the *Salmonella* H antigens are specific only to *Salmonella*. The principle O antigens for *Listeria* are O1, O3, O4a, O4b, and O4d. Only three H antigens are used for serotyping: HA, HC, and HD. All *Listeria* have HB. The serotyping procedure is the tube agglutination as outlined in FDA BAM (1998); and, as in the BAM manual, it is suggested that strains for serotyping be sent to Toxin Technology, Inc, 7165 Curtiss Avenue, Sarasota, FL, 34237. This is an involved procedure but all of the typing antisera can also be purchased directly from Toxin Technology.

Listeria can be classified in a number of subgroups on the basis of their O and H antigens. There are seven major groups, but subgroups 1/2a and 4b most commonly occur in food-poisoning outbreaks. At one time, industry representatives suggested that recalls be based only on these serotypes; however, this was denied by regulatory agencies (see Table 23.3).

Methods for *Listeria*
Listeria FDA BAM Chapter 10, Revision A, 1998 (1998)
Listeria USDA Microbiological Laboratory Guidebook, Chapter 8, 3rd Edition (USDA)
(Other methods for *Listeria* are available, but the reader is cautioned to only use those methods that have been validated against their specific sample matrix. A more complete list of rapid methods is available through AOAC and FDA BAM Supplement 1A and 1B to Chapter 10 *Listeria monocytogenes*, and Appendix 1, Rapid Methods to FDA BAM.)

VEROTOXIGENIC *E. COLI*: *E. COLI* O157:H7
Organism Characteristics

There are various types of pathogenic *E. coli*, but the major type of concern to the dairy industry is the serotype O157:H7. Described by their “O” (somatic) antigen, number 157 and their “H” (flagellar antigen, number 7; it is one of a number of different types of verotoxigenic *E. coli* (VTEC) but certainly one that has been recognized as a serious health risk.

Like *Salmonella*, *E. coli* can have both somatic and flagellar antigens (some strains lack the H antigen). Ecological studies have revealed that the bovine species and to a much lesser extent other ruminants are the reservoirs for this serotype. It is not found only in raw milk and ground beef but also in a wide variety of other food products. Its pathogenicity is associated with the production of two toxins referred to as verotoxins. The latter are serologically related to a toxin produced by *Shigella dysenteriae* and cross-react with antisera to it. The toxins are often referred to as Shiga-like toxins.

Both the USDA and the FDA have a cultural procedure for the isolation of the serotype (FDA BMA, 1998; USDA FSIS Microbiological Laboratory Guidebook). There is an excellent presentation regarding the physiology, serology, and isolation procedures for serotype O157:H7 in the Compendium of Methods for the Examination of Foods (Downes and Kieth, 2001).

Illness

E. coli O157:H7 infections are similar to *Salmonella* in that it produces gastrointestinal symptoms, such as abdominal cramps, diarrhea (often bloody but not necessarily), occasionally vomiting, with little or no fever. The infectious dose may be <100 CFU, with the onset of symptoms occurring an average of 3–8

Table 23.3. Classic Serological Reactions of *Listeria*

Serogroup	O Antigens	H Antigens	Species with Given Serotype
1/2a	I, II	A, B	<i>L. monocytogenes</i>
1/2b	I, II	A, B, C	<i>L. monocytogenes</i> , <i>L. seeligeri</i>
1/2c	I, II	B, D	<i>L. monocytogenes</i>
3a	II, IV	A, B	<i>L. monocytogenes</i>
3b	II, IV	A, B, C	<i>L. monocytogenes</i>
3c	II, IV	B, D	<i>L. monocytogenes</i>
4a	VII, IX	A, B, C	<i>L. monocytogenes</i>
4ab	V, VI, VII, IX, X	A, B, C	<i>L. innocua</i>
4b	V, VI	A, B, C	<i>L. monocytogenes</i>
4c	V, VII	A, B, C	<i>L. seeligeri</i>
4d	VI, VIII	A, B, C	<i>L. seeligeri</i>
4e	V, VI	A, B, C	<i>L. seeligeri</i>
5	VI, IX	A, B, C	<i>L. ivanovii</i>
7	XII, XIII	A, B, C	<i>L. monocytogenes</i>
6a	V, XV	A, B, C	<i>L. welshimeri</i> , <i>L. innocua</i>
6b	IX, X, XI	A, B, C	<i>L. welshimeri</i> , <i>L. innocua</i> , <i>L. seeligeri</i>
	XII, XIV	E	<i>L. grayi</i>
	XII, XIV	E	<i>L. murrayi</i>

Note: 1/2a and 4b are the predominate strains found in food samples involved in outbreaks.

Courtesy of Deibel Laboratories, Inc.

days after consumption of the organism. The duration is typically 2–14 days (6 days on average), with longer potential sequelae such as the hemolytic uremic syndrome seen in some cases. The mortality rate is relatively low unless complications ensue.

Sources

Most vehicles for *E. coli* O157:H7 concern the avoidance of contamination from fecal sources, including water, cattle feces, and person-to-person contact.

Foods Implicated

Raw milk, unpasteurized juices, fresh produce, and raw meats (especially raw ground beef) make up the predominate foods implicated thus far. The organism can also spread via person-to-person contact, in what is vividly described as the “oral–fecal” route of transmission.

Control and Prevention

In diary foods, the same control measures must be taken as in those for *Salmonella* and *Listeria*: thorough cooking of prone products, pasteurization of liquids, high-pressure treatment (applicable to intact product as well as ground products), irradiation, prevention of cross-contamination between raw

and cooked foods, good manufacturing practices surrounding personal hygiene, effective water treatment systems so the plant water is safe, and so on.

Testing Methods for *E. coli* O157:H7 in Dairy Products

The best testing methods are performed by rapid testing assays, especially PCR- and ELISA-based screening systems, as there are strong genetic links that make up the virulence factors for this organism. There are cultural methods using variations of MacConkey (MAC) agar that employ different carbohydrates, such as Sorbitol MacConkey (SMAC) and Tellurite-Cefixime-Sorbito MacConkey (TC SMAC) agars. These cultural assays are very tedious, time-consuming, and often must be repeated several times to get a result, even when dealing with known positive cultures. The main issue with the cultural aspects of testing for this organism is the inability to select against other members of the family *Enterobacteriaceae*, and a lack of distinctive differential components that would allow the microbiologist from distinguishing them on a plate. *Enterobacteriaceae* also hold the genera *Salmonella*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia*, *Shigella*, *Aerobacter*, and so forth.

Certain chromogenic agars are becoming available which show promising results in collaborative studies of their efficacy. In these systems, the target organism is distinguished from competitive flora on the petri plate by a distinctive colony color. For instance, one such media will show *E. coli* O157:H7 colonies as a bright turquoise against a clear background agar, while other *E. coli* that are not O157:H7 will appear white. If these chromogenic medium prove to be reliable in the detection of *E. coli* O157:H7, they could dramatically drive the cost of the assay down, providing the client a cost-effective alternative to the normally expensive PCR or ELISA tests currently preferred by industry.

In the current cultural assay, the sample is homogenized into a general growth media, such as tryptic soy broth (TSB) with the antimicrobics Cefixime, Cefsulodin, and Vancomycin. The homogenate is incubated overnight at 35°C and streaked out onto TC-SMAC, which is also incubated overnight at 35°C. Sorbitol-fermenting bacteria will appear as pink to red colonies; *E. coli* O157:H7 will appear as colorless or neutral/gray colonies, with a smoky center. Pick five typical O157:H7 colonies from TC-SMAC onto a general growth medium petri plate, such as TSA YE, without antimicrobics. Incubate at 35°C for 18–24 hours. These colonies should be tested biochemically to confirm that they are *E. coli*, and can be run on a number of confirmation test kits, such as ELISA- or PCR-based assays. Several are identified on FDA BAM for the exact manufacturers.

Confirmation testing for cultural assays by FDA BAM involves “immunomagnetic” capture of the organisms as a way to filter larger volumes of organisms, designed for better selection of the target organism.

Methods for the Detection of *E. coli* O157:H7

E. coli O157:H7 FDA BAM, Chapter 4a, Revision A, 1998 (FDA BAM, 1998)

E. coli O157:H7 USDA Microbiological Laboratory Guidebook, Chapter 5, 3rd edition (USDA)

(Other methods for *E. coli* O157:H7 detection are available, but the reader is cautioned only to use those tests that have been validated against their specific sample matrix.)

S. AUREUS

S. aureus is unlike other dairy pathogens in that there are several key factors that must be present for this bacterial pathogen to pose a risk to the consumer. Not all *Staphylococci* have the ability to be food-borne

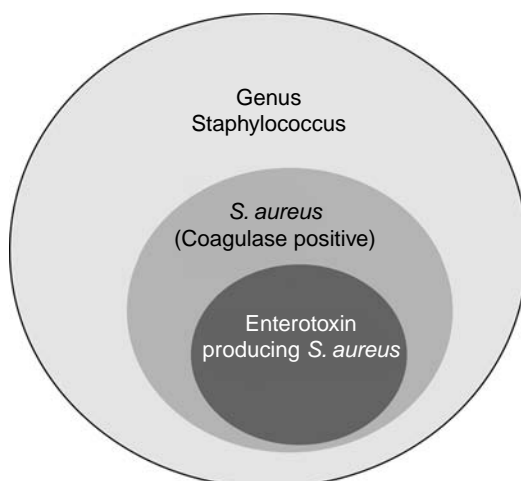


Figure 23.30. Diagrammatic representation of *Staphylococcus* genus and enterotoxigenic strains. As seen above, all *S. aureus* that produce toxin also produce the coagulase enzyme; however, not all *S. aureus* strains are toxin producers. This relationship serves as a relatively quick and cost-effective means for testing for toxin producers by identifying only coagulase positive strains and obviating the need for expensive toxin testing. (Figure courtesy of Deibel Laboratories, Inc.)

pathogens, only certain ones in the species *aureus*. All *S. aureus* are pathogens, including those that are capable of producing a bacterial enterotoxin, called *S. aureus* enterotoxin (SET). This enterotoxin is only produced when the capable *S. aureus* in a food sample reach a level of around 1×10^5 ; generally this only happens if the food product is temperature abused, allowing for the growth of this pathogen. The majority of the pathogenic *S. aureus* exhibit an interesting biochemical trait: all produce coagulase that will react with rabbit plasma EDTA forming a gel “clot” (Figs. 23.30 and 23.31). Various surveys have reported a range of 50–70% of coagulase-positive *S. aureus* produce the enterotoxin. This biochemical trait of coagulase production provides an easy and cost-effective means of screening cultures for potential toxin producers (Figure 23.30).

S. aureus possess a wide variety of virulence factors including a number of serologically different enterotoxins. The production of enterotoxin is a coincidence of growth. The principal target for the enterotoxins is humans and aside from other primates other animals are not affected. This toxin is extremely heat-stable. In contrast to the heat-sensitive vegetative cell,

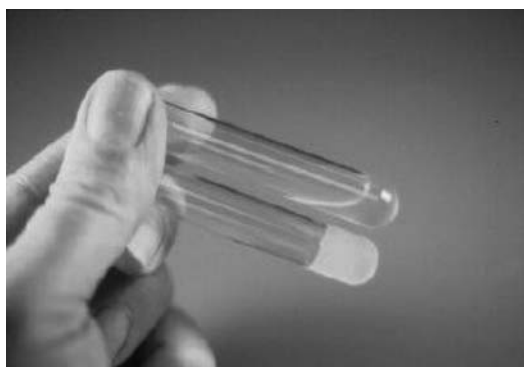


Figure 23.31. Coagulase test for *S. aureus* detection. Top tube = coagulase negative reaction, bottom tube = coagulase positive reaction. A firm clot is necessary to be considered a positive test. (Picture courtesy of Deibel Laboratories, Inc.)

the enterotoxin withstands thermal processes significantly higher than those used for milk pasteurization. Although *S. aureus* is involved in some mastitis occurrences, it is not a problem and it does not grow to significant numbers in raw milk unless the milk is mishandled. The organism is readily killed by normal pasteurization processes.

Generally, the enterotoxin producing strains associated with humans produce enterotoxin serotype A. This is reflected in outbreak data in that in most outbreaks enterotoxin-A-producing strains are involved. Thus, the human food handler and subsequent temperature abuse of the product are usually associated with outbreaks.

Many products using manufacturing grades of milk employ a thermal process in which the finished product is referred to as “heated milk.” This is a subpasteurized product and it is more prone to the growth of *S. aureus* if not handled properly. In cheese making, “slow starters” can be due to bacteriophage or antibiotics and allow the growth of *S. aureus*. With dried dairy products (i.e., dried milks, whey, buttermilk, and cheeses), temperature abuse of the raw milk and/or improper temperature control during the early stage of drying has been associated with food-poisoning outbreaks. Additionally, improper temperature control of the cream used in butter manufacturing and a low salt content of the product will allow the growth for *S. aureus*.

There are multiple formats for detecting the preformed toxin, but the various ELISA formats are fast and very reliable. However, cost is an issue thereby

limiting these assays to mainly just confirmation of toxin producers from samples with high-coagulase positive *S. aureus* counts. The toxin accumulates in the food matrix and is extracted usually with an acidified aqueous solution. Depending on the method employed (kits are available and should be followed per the directions manual), a result can be obtained in a few hours. Usually enterotoxins A through E are detected.

Organism Characteristics

S. aureus are Gram-positive, nonsporeforming cocci, and are facultative in their oxygen requirements. They are ubiquitous, occurring in a large percentage of the human population. Enterotoxigenic strains are usually (but not always) positive for the coagulase enzyme.

Illness

Typical gastrointestinal intoxications (vomiting, nausea, abdominal cramps, diarrhea, and acute prostration) are the symptoms caused by the preformed toxin. Growth is always required in order to obtain high enough populations (millions) to yield detectable toxin levels, or 100,000/g in the product. Usually, a person must ingest >200 ng toxin (0.1 to 1 µg/kg body weight) to become ill, with the onset of illness usually 1–7 hours (usually 2–4 hours on average) after consumption of the toxin. The duration of the illness is usually 24–48 hours, with a very low mortality rate. Illness caused by this toxin generally leaves a lasting memory on the person affected, as it can be a very painful experience.

Numerical data must be interpreted with caution when considering the safety of a product. For instance, an *S. aureus* count of 1,000/g is meaningless unless the total history of the product is known and even then it may be subject to error. The theoretical relationship between time, count, and toxin production is considered in Figure 23.32. A low count at the end of the *S. aureus* growth period is misleading as maximum toxin production has occurred previously. If in doubt, this consideration can be obviated by a direct test for the enterotoxin.

Sources

Animals or humans are the sources of the pathogenic *S. aureus*; however, the latter pose the largest risk to the consumer. Humans are the main source of enterotoxigenic strains; nasal passages, skin, hair, oils, pimples, and infected wounds. It constitutes part of

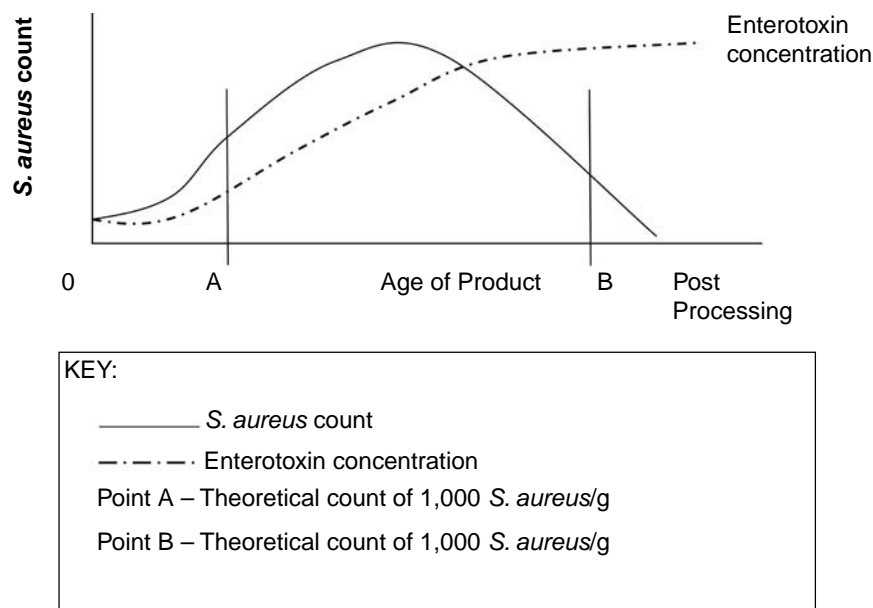


Figure 23.32. Theoretical growth of *S. aureus* and the production of toxin in a product. (Diagram courtesy of Deibel Laboratories, Inc.)

the normal skin flora it is a permanent resident of the upper respiratory tract, and it is found throughout the alimentary canal.

Foods Implicated in Illness

Foods handled after thermal processing can be contaminated by the food handler. If the food is subsequently temperature-abused (not held under proper refrigeration), *S. aureus* may grow to high numbers and produce toxin in the food.

Staphylococcal intoxications may also be caused by improperly prepared dried powders. *S. aureus* is resistant to drying and can survive high salt levels. Unless proper fermentation procedures are used, *S. aureus* can grow to high levels during the fermentation of cheese and sausage products like summer sausage or dry salami.

Control and Prevention

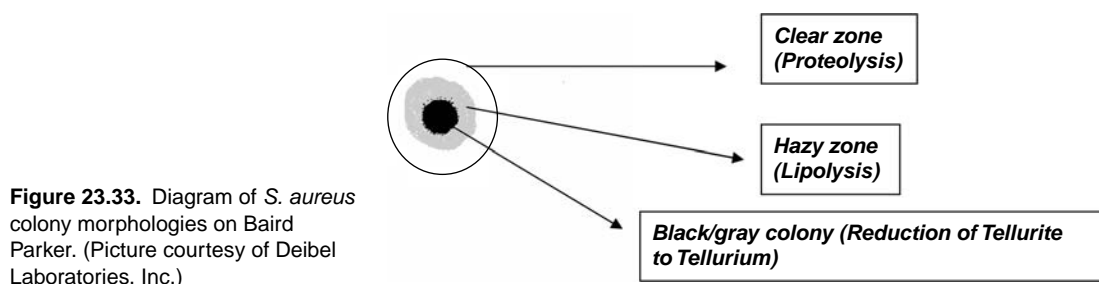
Most control practices revolve around routine current good manufacturing practices for personal hygiene, as well as prompt and proper refrigeration of foods and food ingredients to prevent the growth of the organism. Food handlers should avoid touching

the nose and mouth, should wash their hands with a bactericide, and must cover all wounds properly.

Testing Methods for *S. aureus*

On the basis of the FDA BAM methodologies, decimal dilutions of sample homogenates are “spread plated” onto three pre-poured Baird Parker (BP) petri plates; 1.0 mL is aseptically aliquoted onto three BP plates, 0.3 mL, 0.3 mL, and 0.4 mL, respectively. The samples should be immediately spread onto the entire plate, starting from the highest dilution to the lowest, using sterile bent glass rods (“hockey sticks”), or similar. This obviates the sample homogenate from being soaked up into the agar medium before it can be evenly distributed.

Coagulase and noncoagulase producing strains may be represented on the same plate, or in the same sample homogenate. To obtain accurate counts, count each different colony morphology on the BP plates and record the results. Subject two to five colonies of each morphological type to coagulase testing, by picking into sterile 10 mL test tubes of brain heart infusion (BHI) broth. Incubate the BHI cultures 18–24 hours at 35°C. Subject each BHI tube to coagulase testing and obtain the count per gram by



multiplying the fraction of coagulase-positive cultures to the colony morphology count on the plates (see Figs. 23.33 and 23.34).

Methods for Detection of *S. aureus*

S. aureus FDA BAM Chapter 12, Revision A, 1998 (1998)

(Other methods for *S. aureus* are available, but the reader is cautioned to only use those methods that have been validated against their specific sample matrix.)

SPRAYING FOR CONTROL OF *SALMONELLA* AND *LISTERIA* IN DAIRY PLANTS USING BACTERIOPHAGES

As bacteria can enter into the product stream and contaminate a lot of production, so can a specific bacterial strain enter into a dairy facility and become entrenched in equipment and the environment. This

organism can survive indefinitely and will occasionally enter into the product stream, causing downtime and a loss of revenue. The authors generically refer to this as a facility's "house pet."

In the past, the only way to rid some plants of a house pet is to tear the plant down and start over. When considering a *Salmonella* house pet, the organism becomes pervasive in small niche environments of the processing equipment and facility grounds, and eradication is extremely difficult. At best, sanitation practices may make control of the organism feasible, but if there is any lax or lessening up, the organism will become a problem.

Every living cell on the planet, prokaryotic and eukaryotic alike, have the ability to become infected by a virus specific to that cell. The authors have taken this fact of science and utilized this set of circumstances to rid dairy plants of *Salmonella* house pets where the plant has a single strain of *Salmonella* in the

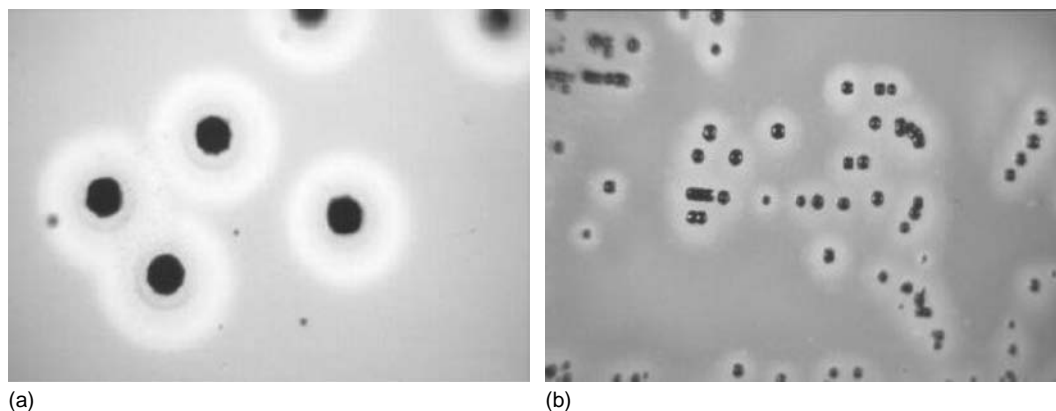


Figure 23.34. Picture of typical *S. aureus* colonies on Baird Parker. *Staphylococcus aureus* on Baird Parker agar; note the lipolytic and proteolytic zones surrounding black colonies. (Picture courtesy of Deibel Laboratories, Inc.)

plant environment. In this scenario, the *Salmonella* strain if not controlled will get into the food stream and contaminate the product.

Salmonella phages, specific to a strain of *Salmonella*, have been actively used for eradicating a dairy facility of a house pet scenario. *Salmonella* phages, with a few exceptions, are strain specific and a phage can be isolated for just about any strain. Once isolated, the phage can be grown to extremely high titers, concentrated, and used to treat the infected plant. Over 15 plants have been treated in this manner with excellent results, and a low incident of reoccurrence of the target pathogen.

At first regulatory official looked upon this procedure with disdain claiming it obviated sound sanitation practices. However, in recent times, they have promulgated the use of phage control of microorganisms including *Listeria sp.* in various food plants, and phage preparations for *Listeria* control are commercially available. Strain specific for *Listeria* can be overcome when dealing with this genus.

PROBIOTICS

ENUMERATION OF PROBIOTICS AND SHELF STABILITY

With the popularity of Probiotics, many producers now seem to be throwing in “everything but the kitchen sink” such as numerous other species of bifidobacteria and lactic acid bacteria (LAB). Some producers are even adding strains of bacillus, or even nonviable bacteria into their products. So far there is no scientific evidence that any health benefits result in using these bacterial strains, but to say the industry is booming would be a gross understatement. In a recent publication in the *Journal of Food Science* (2007), researchers were identifying enhanced isolation techniques for *Lactobacillus* species, for use in pet-care products for dogs!

It should be stressed that two important factors must be considered when identifying a new species for probiotic use: (1) scientifically generated data identifying a defined healthful benefit to the host for a given species or strain and (2) the dose requirement needed to insure the healthful benefit is seen in the host. The second question is of vital importance to both the consumer and to the manufacturer, as any health benefit claim will only be seen if the organism can reach the small intestine of the host and at high enough representation. This is a numbers game.

Probiotic cultures are picked by their ability to survive within a host system, battling their way through the salivary enzymes, stomach acids, bile salts in the small intestine, and anaerobic growth environment. The cultures must offer a healthful benefit to the host upon their transient colonization in the lower small intestine and the large intestine. At each stage in the digestion process, a single dose of a probiotic culture will experience some die-off of its numbers. It is imperative that the dose remains high enough such that enough of the probiotic culture remains viable for their short-term colonization. If the numbers that colonize are not high enough, no healthful benefit will be obtained by the host.

Probiotic cultures unfortunately lack the ability to survive indefinitely in the host. This is good for manufacturers as the food products will continuously need to be ingested for the healthful benefit to remain. One drawback is that the healthful benefit will not be seen for a few weeks, as it takes this long for large enough numbers to remain viable in the intestine. This concept is called transient colonization and is roughly defined as an organism's ability for short-term survival before they are flushed out of the host's intestine.

From a bacterial perspective, the intestinal track can be viewed as high-end real-estate offering bacteria nutrients, a consistent temperature range for survival, and plenty of moisture for growth. The offset is that this environment is highly competitive, with organisms developing survival mechanisms to outgrow their competitors for long-term colonization. An example would be the production of bacteriocins to kill other organisms, explosive growth rates to secure an open niche, and attachment mechanisms to rigorously attach to the intestinal wall.

Issues in Culturing

Multi organism probiotic cultures make it difficult if not impossible, to separate out a certain species by cultural tests at the laboratory level. This is especially true for different species of the same genus that are included in a product, such as different *Lactobacillus* species. The best techniques for this identification is by direct DNA typing, but cost and speed to result are critical factors to be taken into account, which do not make this a rather viable option at this time.

Organisms from the same genus are not different enough physiologically or culturally to be able to separate and enumerate them. Another limitation is the inability to obtain the most accurate count for a

given organism when the individual organism cannot be grown under its optimum conditions due to interference from another culture(s) that was also added.

In most of the food industry, the three most important factors that must be addressed at the laboratory level are cost, time-to-result, and data quality, with generally only two of the three being possible! The consumers can be given a fast and accurate result, but at a higher cost, or they can receive a fast result at a fair price, but the quality of the assay might not be as good as other methods available to the laboratory. Conversely, there seem to be only one factor that must be addressed by the laboratory for enumerating probiotic cultures: can your laboratory obtain the desired count?

Commonly, laboratories are asked to enumerate *Lactobacillus* (acidophilus) and *Bifidobacterium* (infantis) as they are the two predominate probiotics used in the food industry. The *Lactobacillus* will be able to grow both aerobically and anaerobically. The *bifidobacterium* are strict anaerobes and are able to grow only anaerobically. Therefore, to accurately obtain the *bifidobacterium* count, it is necessary to perform two tests on the same sample: an aerobic count that should enumerate just the *lactobacilli*, and an anaerobic count that should enumerate both *lactobacilli* and *bifidobacteria*. To obtain the *Bifidobacteria* count, subtract the aerobic count (*lactobacilli* only) from the anaerobic count (*lactobacilli* and *bifidobacteria*). This is a good example of a limitation. This procedure is not optimum for the recovery of the *Lactobacillus* since it would grow better anaerobically and with the addition of cysteine, an oxygen scavenger aiding in maintaining an anaerobic environment.

Since the laboratory can only obtain the *bifidobacteria* count by default, arguably this would appear inflated. This procedure is commonly classified as a "differential count" and is a less than ideal method of enumerating these two bacterial species in a sample.

A problem that arises for laboratories is the testing methods are specific to the manufacturer, and not routinely available through AOAC or FDA BAM. Manufacturers do spend considerable time developing optimal methods for enumerating their organism on the basis of the specific stabilization practices they have developed. These plant processes are often highly proprietary and the methods are generally only optimal when testing the bulk raw material. Once the probiotic culture is incorporated into a food matrix, the manufacturer's method may not be ideal for obtaining

accurate counts, as intrinsic factors of the product, and background microflora play a role in the enumeration procedure.

There are many methods available to the laboratory for making an informed decision as to the best method for counting the organism(s). When time to result and obtaining correct "high counts" are of prime importance, the laboratory can be at a disadvantage without being given the time to research the best method for culturing and counting.

At this point, the best advice that can be given to the laboratory is along the lines of formulating validation testing, to be conducted on trifold concerns of the food matrix, the probiotic manufacturer, and the stabilized probiotic culture. In this way, several methods can be evaluated against a single sample for the best method to be used. Both individual organism cultures can be enumerated as well as multiculture cocktails with the food matrix, again with the goal of obtaining the best method specific to the probiotic manufacturer, the probiotic culture used, and the food matrix it is incorporated into.

Probiotic Testing Methods

APT and MRS media provide the foundation for culturing and enumerating the probiotic (lactic acid bacteria) organisms used by industry. Both have been slightly augmented to provide better recovery of the organism by probiotic manufacturers as well as academics for research purposes.

Enumerations: *Bifidobacterium*

Procedure. If the sample contains only *Bifidobacterium*, make a 1:10 dilution in MRS broth, stomach for 30 seconds. *Bifidobacterium* are strict anaerobes so stomach briefly obviating the incorporation of oxygen. This is allowed to rehydrate for 30 minutes (room temperature). Perform dilutions in triplicate using a micropipettor and factory made 99 mL diluents. Plate with MRS + cysteine-HCl. The final cysteine concentration should be 0.05% (add 1.0 mL of a 5% cysteine-HCl filtered-sterilized solution to each 100 mL of MRS agar).

Enumerations: *Lactobacillus*

Procedure. If the sample contains only *Lactobacillus* follow the above procedure but stomach for 2 minutes to insure proper homogenization.

Enumerations: Total Counts of *Bifidobacteriu* and *Lactobacillus*

Procedure. If the sample contains both *Bifidobacterium* and *Lactobacillus* and do not need separate counts for each of the organisms, follow the first procedure (“*Bifidobacteriu* Only” procedure as in above—stomaching for only 30 seconds).

Enumerations: Differential Counts of *Bifidobacteriu* and *Lactobacillus*

Procedure. If the sample contains both *Bifidobacterium* and *Lactobacillus* and separate counts are needed, perform a differential count. Weigh out two separate 11 g samples.

1. Rehydrate the *Lactobacillus* in APT broth (not MRS). Stomach for 2 minutes. Rehydrate for 30 minutes at room temperature. Plate in APT (no cysteine). Cap with more of the same and incubate aerobically at 38°C for 72 hours.
2. For the *Bifidobacterium* rehydrate in MRS broth and stomach only for 30 seconds. Plate in MRS + cysteine. Cap with more of the same and incubate anaerobically at 38°C for 72 hours.
3. (Differential count) The *Lactobacillus* count is just the count obtained from the APT aerobic plates. The *Lactobacillus* will be able to grow both aerobically and anaerobically. Both organisms will be counted in the MRS + cysteine, so subtract the APT count to obtain the *Bifidobacterium* count. This procedure will more than likely give false-low *Lactobacillus* counts, and false-high *Bifidobacteriu* counts.

Methods

Lactic Acid Bacteria, Compendium for the Microbiological Examination of Foods, 4th edition (Downes and Kieth, 2001)

Enumerations: Activity Testing In Yogurt

“Yogurt,” (refrigerated) by definition must contain both *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. All yogurts are required to be made with these two organisms, but may contain other (nonprobiotic) cultures additionally. No definition standard exists for frozen yogurt cultures (as it may or may not contain active cultures).

To qualify for the NYA (National Yogurt Association) seal, refrigerated yogurt must contain a minimum of 100 million CFU/g at the time of manufacture

and frozen yogurt must contain at least 10 million CFU/g at the time of manufacture.

Procedure. *L. delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*)—Plate in acidified MRS (acetic acid) and incubate anaerobically at 37°C for 72 hours.

Streptococcus thermophilus—Plate in M17 (add lactose solution) and incubate aerobically at 37°C for 48 hours.

Initially, yogurt samples are plated for the two organisms at the “Beginning,” “Middle” and “End” of production. The combined count for both organisms (for each testing period—B, M, and E) should be 10^8 . At the end of the yogurt’s stated shelf life, the activity testing can be initiated. A 12% solution of nonfat dry milk is pasteurized at 92°C for 7 minutes. After it is cooled to 43°C, the yogurt under test is inoculated at a level of 3%. A sample is plated for the two organisms initially (“before fermentation count”). The nonfat dry milk culture is then placed in a 43°C water bath for 4 hours. The sample is plated again for both organisms (“after fermentation count”). The laboratory should do platings for probiotics in duplicate or triplicate, using micropipettors and factory made blanks. This limits the error of the assay, and increases the data quality and integrity of the assay.

Cultures must be active at the end of the stated shelf life (determined by the Activity Test) and two of the three samples must show an increase of at least one log during fermentation.

Methods

Activity Testing in Yogurt, National Yogurt Association, (2007)

ELISA TESTING FOR PATHOGENS AND TOXINS

In many of the assays for pathogens in dairy products, a screening procedure involving immunological principles is used. The procedures are abbreviated as EIA, ELISA, or ELFA for enzyme-linked-immunosorbent assay. The basic scheme involves “sandwiching” of antibodies to a target antigen. The primary antibody (sometimes called the capture antibody) is attached to the plastic microwell using plastics that have an affinity for proteins. The enriched sample is added followed by a thorough washing to remove extraneous material. To the fixed antigen-antibody complex, a second antibody containing a “flag” or signal molecule is added (horseradish peroxidase or a fluorescent dye are usually used). Subsequently, the

indicator is developed and read spectrophotometrically (peroxidase) or depending on the “flag” using other instruments (see Fig. 23.35).

Limitations of ELISA-Based Screening Assays

These screening procedures require verification of “positive” results using traditional cultural methods. A “positive” ELISA result can be a false-positive and confirm as negative after cultural-biochemical testing, whereas a “negative” ELISA result is usually accepted as such without further testing.

It is entirely possible that a substance can bind to the capture antibody bound in the well that is not the target antigen. If this antibody is not specific to that substance and it reacts with the capture antibody, it will not be washed out of the well. This is called nonspecific antigen binding, and will result in a false ELISA positive.

Many times there are chemicals inherent to the food matrix that can form a nonspecific antigen binding. An example of this could be the chemical-smoking agent used in some meat and cheese operations. These types, as well as many others, may result in a false ELISA positive.

The most common types of ELISA positives that are confirmed negative by further testing are those associated with other bacteria. Many target organisms share similarities (such as somatic or O-antigens) with closely related organisms that can yield false ELISA positive results. An example of this similarity can also be similar biochemical pathways between closely related genera of organisms, such as members of the family *Enterobacteriaceae*, which routinely present false positives for *Salmonella* by standard ELISA testing.

PHAGE CONCERNS

Bacteria can be infected by viruses and are referred to as bacteriophages, or just phages for short. For the most part, the phages are not specific to a genus or even species-specific but usually strain-specific. Dairy plants using starter cultures can experience a phage infection wherein the fermentation is “slow” or fails completely. Continued use of the same started culture strain increases the probability of this infection and avoidance entails a rotation of the starter strains. This is a common practice in dairy plants using starter cultures.

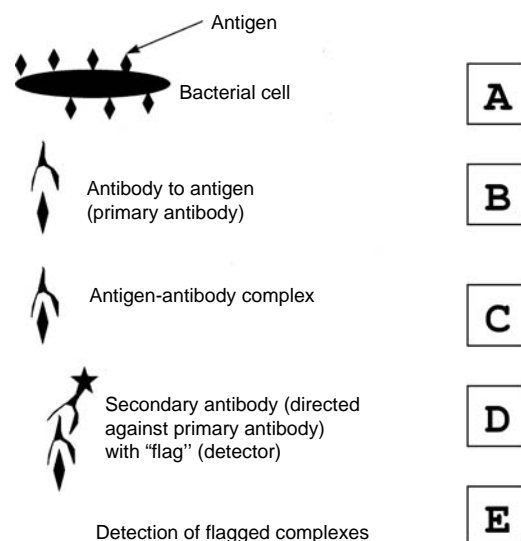


Figure 23.35. Basic principle of ELISA—The “Sandwich.” (Figure courtesy of Deibel Laboratories, Inc.) (A and B) Most Bacteria have “antigenic sites” covering the outside of their cell walls. An antigenic site is anything that is capable of eliciting an immune response. This is why antibodies can be tailored to bind to these antigenic sites; it is a natural way for humans and animals to identify foreign matter in the host. A Well within the test kit has bound antibodies on its surface. These antibodies are specific for a target organism or genus. For example, many ELISA manufacturers now have both a genus *Listeria* kit and a *Listeria monocytogenes* specific kit. (C) An enriched sample is transferred into a well containing the bound antibodies. Only an organism with specific antigenic sites will bind to the antigen-specific antibody. (D) The well is washed to remove unbound substances that are not the target organism. More antibodies that are also specific to the target organism are added to the well. These second antibodies are actually bound to a second agent (usually an enzyme). (E) From here, the well is washed again to remove any unbound substances. Finally, a substance (sometimes an enzyme to activate a fluorescent component, or a substrate to activate a bound enzyme) is added to activate the bound component. This activated component is detected in the diagnostic instrument, which records that the sample is “positive” for the target organism, by changing color in a colorimetric assay, by fluorescing in a fluorescence assay, or by producing light in a bioluminescence assay. It should be mentioned that this results in a presumptive, and must be confirmed by biochemical testing such as those described in FDA BAM or USDA FSIS methods.

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