DAIRY TECHNOLOGY

Principles of Milk
Properties and Processes

○ submicelle

protruding
peptide chain

· calcium
phosphate

50 nm

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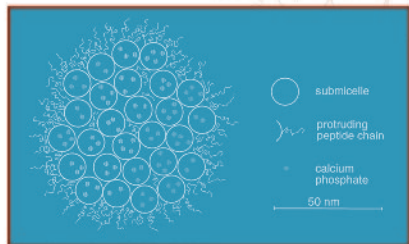# DAIRY
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Principles of Milk
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FOOD SCIENCE AND TECHNOLOGY

A Series of Monographs, Textbooks, and Reference Books

Preface

The primary theme of this book is to present the efficient transformation of milk into high-quality products. The changes in raw material and intermediate and final products and the interactions between product and process equipment are covered most prominently. Thus, it especially treats aspects that are specific for milk processing with the advanced dairy industry in mind. The stress is on principles of physical, chemical, enzymatic, and microbial transformations. Detailed manufacturing prescriptions or product specifications are not given, since they are widely variable.

Aimed at university food science and technology majors, the book is meant as a text, although it may also be useful as a work of reference. It is assumed that the reader is reasonably familiar with the general principles of food chemistry, microbiology, and physics and with elementary food engineering. In some instances, general aspects are briefly recalled. The book contains no references to the literature, but every chapter ends with suggestions for further reading. Most of the tables and figures were newly created, but for those that are reprinted a reference is given.

The book is made up of four parts. Part I, ''Milk,'' discusses the chemistry, physics, and microbiology of milk. This provides the basis for understanding what happens during processing and storage. Part II, ''Processes,'' details the main unit operations applied in the manufacture of milk products. These are treated in some detail, including the influence of product and process variables on the result. Some general aspects of processing are also discussed. Some highly specific processes, such as churning, are discussed in product chapters. In Part III, ''Milk Products,'' examples of the integration of knowledge of the raw mate-

rial and of processing in the manufacture of products are discussed. The procedures needed to ensure consumer safety, product quality, and efficient processing are treated. The list of dairy products is almost endless and some groups have been selected because of their general importance or to illustrate relevant aspects. Finally, Part IV, ''Cheese,'' describes the processes and transformations (physical, biochemical, and microbial) in the manufacture and maturation of cheese. Here the processes are so specific and the interactions so intricate that a separate and integrated treatment is needed. This part starts with generic aspects and then discusses some specific groups of cheeses.

The nucleus of this book was a series of lecture notes (in Dutch) from Wageningen Agricultural University on dairy science and technology. Although meant for students, these lecture notes, of which several editions have been issued, were also frequently used by food technologists in practice. Hence, it was thought worthwhile to produce an international edition. The lecture notes were combined, reorganized, partly rewritten (to make them more internationally useful), and translated into English to result in one integrated textbook.

There was considerable overlap between part of the said lecture notes and the book by P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (John Wiley and Sons, 1984). A similar overlap occurs between the latter book and this one, part of which can be seen as an update of *Dairy Chemistry and Physics.* We are greatly indebted to Wiley, and to Dr. R. Jenness, for allowing us to draw heavily on that book.

Several colleagues, too many to name them all, have been helpful in providing specific knowledge or in scrutinizing part of the book. Besides the important contributions by Dr. Jenness, we want to mention the late Professor E. A. Vos, who made the first versions of some of the lecture notes; Professor M. G. van den Berg, who commented on parts of the book; several colleagues from the Netherlands Institute for Research in Dairying (NIZO) who provided specific information; and, finally, all the people of our department who helped in various ways and especially for cooperating in all the research that was aimed at enhancing our understanding of the principles of dairy technology.

All the authors of this book contributed to the original lecture notes and scrutinized parts of the draft for this book. Dr. T. J. Geurts made the English translations. The undersigned took responsibility for the final organization and editing of the book.

P. Walstra

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Units and Conversion Factors

Throughout this book, SI units are used. For the convenience of some readers, some conversion factors are given below.

1 bar	$= 0.987$ atm $= 14.5$ psi $= 10^6$ dyne/cm^2 $= 10^5$ Pa
1 cal	$= 4.18$ J $= 0.00397$ BTU
1 erg	$= 10^{-7}$ J
1 erg/cm^2	$= 1$ mJ $\cdot$ m^{-2}
1 cP	$= 1$ mPa $\cdot$ s
1 dyne/cm	$= 1$ mN $\cdot$ m^{-1}
%	$=$ percent by weight, unless stated otherwise

I

MILK

1

Composition, Structure, and Properties

1.1 COMPOSITION AND STRUCTURE

This section is essentially an introduction to the chemical components and structural elements of milk. A fuller understanding will be reached through some of the ensuing chapters, where most subjects are discussed in greater detail.

1.1.1 Principal Components

A classification of the principal constituents of milk is given in Table 1.1. The principal chemical components or groups of chemical components are those present in the largest quantities. Of course, the quantity (in grams) is not paramount in all respects. For example, vitamins are important with respect to nutritive value; enzymes are catalysts of reactions; and some minor components contribute markedly to the taste of milk. More information on milk composition is given in Table 1.3.

Lactose is the distinctive carbohydrate of milk. It is a disaccharide composed of glucose and galactose. Lactose is a reducing sugar.

The *fat* is largely made up of triglycerides, constituting a very complicated mixture. The component fatty acids vary widely in chain length (from 2 to 20 carbon atoms) and in saturation (0 to 4 double bonds). Other lipids that are present include phospholipids, cholesterol, free fatty acids, and diglycerides.

About four-fifths of the *protein* consists of casein, which in turn is a mixture of approximately 10 different proteins. The remainder is mainly made up of the so-called serum proteins, in addition to several proteins negligible in weight, such as enzymes.

TABLE 1.1   Approximate Composition of Milk[1]

Component	Average content in milk (% w/w)	Range[2] (% w/w)	Average content in dry matter (% w/w)
Water	87.1	85.3–88.7	
Solids-not-fat	8.9	7.9–10.0	
Fat in dry matter	31	22–38	
Lactose	4.6	3.8–5.3	36
Fat	4.0	2.5–5.5	31
Protein[3]	3.25	2.3–4.4	25
casein	2.6	1.7–3.5	20
Mineral substances	0.7	0.57–0.83	5.4
Organic acids	0.17	0.12–0.21	1.3
Miscellaneous	0.15		1.2

[1] Typical for milks of lowland breeds.
[2] These values will rarely be exceeded, e.g., in 1% to 2% of samples of separate milkings of individual cows, excluding colostrum and milk drawn shortly before parturition.
[3] Nonprotein nitrogen compounds not included.

The *mineral substances* are not equivalent to the salts. It mainly concerns K, Na, Ca, Mg, Cl, and phosphate. Milk contains numerous other elements in trace quantities. The salts are only partly ionized. The *organic acids* occur largely as ions or as salts; citrate is the principle one. Furthermore, milk has many *miscellaneous components*, often in trace amounts.

The total content of all substances except *water* is called the content of dry matter. Furthermore, one distinguishes solids-not-fat and the content of fat in the dry matter.

1.1.2   Structure

Structure can be defined as the physical arrangement of the (chemical) components in a system. In other words, composition accounts for *what* is in the system, structure *how* it is present. To formulate it in the negative, structure is all that is needed, besides composition and external conditions, to determine the properties of a system.

Figure 1.1 shows the main structural elements of milk. Of course, the picture is schematic and incomplete. Some properties of the structural elements are given in Table 1.2, again in a simplified form; the numerical data mentioned are meant only to define orders of magnitude. The table clearly shows that aspects of colloid chemistry are essential for understanding the properties of milk and the many changes that can occur in it. All particles exhibit Brownian motion;
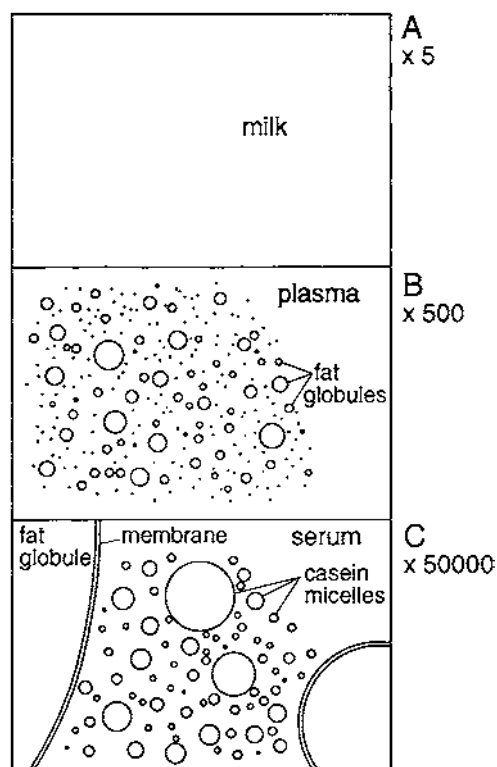
FIGURE 1.1 Milk viewed at different magnifications. The picture indicates the relative size of structural elements. (A) Uniform liquid. However, the liquid is turbid and thus cannot be homogeneous. (B) Spherical droplets, consisting of fat. These globules float in a liquid (plasma), which is still turbid. (C) The plasma contains proteinaceous particles, which are casein micelles. The remaining liquid (serum) is still opalescent, so it must contain other particles. The fat globules have a thin outer layer (membrane) of different constitution. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

they have an electrostatic charge, being negative at the pH of milk. Their total surface area is large.

Fat globules. To a certain extent, milk is an oil-in-water emulsion. But the fat globules are more complicated than emulsion droplets. Especially the surface layer or *membrane* of the fat globule is not an adsorption layer of one single substance, but consists of many components; its structure is complicated. The mass of the membrane is about 2% of that of the fat. A small part of the lipids of milk is found outside the fat globules. Not all of the fat in the globules is liquid; a part of it can crystallize.

TABLE 1.2 Properties of the Main Structural Elements of Milk

	Milk		Plasma	
				Serum
	Fat globules	Casein micelles	Globular proteins	Lipoprotein particles
Main component	Fat	Casein, water, salts	Serum protein	Lipids, proteins
To be considered as	Emulsion	Fine dispersion	Colloidal solution	Colloidal dispersion
Content (% dry matter)	4	2.8	0.6	0.01
Volume fraction	0.04	0.1	0.006	10^{-4}
Particle diameter[1]	0.1–10 μm	20–300 nm	3–6 nm	10 nm
Number per ml	10^{10}	10^{14}	10^{17}	10^{14}
Surface area (cm^2/ml milk)	700	40000	50000	100
Density (20°C; kg · m^{-3})	920	1100	1300	1100
Visible with	Microscope	Ultramicroscope		Electron microscope
Separable with	Milk separator	High-speed centrifuge	Ultrafiltration	Ultrafiltration
Diffusion rate (mm in 1 h)[1]	0.0	0.1–0.3	0.6	0.4
Isoelectric pH	~3.8	~4.6	4–5	~4?

[1] For comparison, most molecules in solution are 0.4–1 nm diameter, and diffuse, say, 5 mm in 1 h. 1 mm = 10^3 μm = 10^6 nm = 10^7 Å.
Note: Numerical values are approximate averages.

Milk minus fat globules is called *milk plasma*, i.e., the liquid in which the fat globules float.

Casein micelles consist of water, protein, and salts. The protein is casein. Casein is present as a caseinate, which means that it binds cations, primarily calcium and magnesium. The other salts in the micelles occur as a calcium phosphate, varying somewhat in composition and also containing a small amount of citrate. This is often called colloidal phosphate. The whole may be called calcium-caseinate/calcium-phosphate complex. The casein micelles are not micelles in the colloid-chemical sense, but just ''small particles.'' They are not homogeneous but are built of smaller particles. The micelles have an open structure and, accordingly, contain much water, a few grams per gram of casein.

Milk minus fat globules and casein micelles is called *milk serum*, i.e., the liquid in which the micelles are dispersed.

Serum proteins are largely present in milk in molecular form or as very small aggregates. Naturally, they bind counterions and a little water.

Lipoprotein particles, sometimes called milk microsomes, vary in quantity and shape. Presumably, they consist of remnants of mammary secretory cell membranes. Few definitive data on lipoprotein particles have been published.

Cells, i.e., leukocytes, are always present in milk. They account for about 0.01% of the volume of milk of healthy cows. Of course, the cells contain all cytoplasmic components such as enzymes. They are rich in catalase.

Table 1.3 gives a survey of the average composition and structure of milk.

1.2 SOME PROPERTIES

Milk is a dilute aqueous solution and behaves accordingly. Polar substances dissolve well in milk; the dielectric constant of milk is high. The viscosity is low, about twice that of water. The substances dissolved in water give milk a certain osmotic pressure (about 7 bar) and freezing point depression (about 0.53 K); the ions present are responsible for the electrical conductivity of milk. The pH is about 6.7. The water activity is high, about 0.993. None of these properties prevents microorganisms from growing in milk. For many microorganisms milk is an almost ideal substrate (also with respect to its composition), although its oxygen pressure is fairly high.

The flavor of milk is discussed in Section 2.6.

Milk is a dilute emulsion of fat in milk plasma. All reactions of other milk components with the fat must occur across the fat globule membrane. Concentrating the fat globules can simply be done by creaming or centrifugation. These processes yield skim milk and cream.

Likewise, the casein can be readily separated from milk, though high-speed centrifugation is needed to separate the micelles centrifugally. After renneting, the micelles flocculate to form a gel that retains most of the fat globules. The

TABLE 1.3 Composition and Structure of Milk[1]

FAT GLOBULE

Glycerides	
triglycerides	40 g
diglycerides	0.1 g
monoglycerides	10 mg
Fatty acids	60 mg
Sterols	90 mg
Carotenoids	0.3 mg
Vitamins A, D, E, K	
Water	60 mg
Others	

CASEIN MICELLE

Protein	
casein	26 g
proteose peptone	+
Salts	2 g
Ca	850 mg
phosphate	1000 mg
citrate	150 mg
K, Mg, Na	
Water	~ 80 g
Enzymes	
lipase	
plasmin	

MEMBRANE

water	+
protein	350 mg
phospholipids	230 mg
cerebrosides	30 mg
glycerides	+
fatty acids	15 mg
sterols	15 mg
other lipids	
enzymes	
alkaline phosphatase	
xanthine oxidase	
many others	
Cu	4 µg
Fe	100 µg

LEUKOCYTE

Many enzymes
e.g., catalase
Nucleic acids
Water

LIPOPROTEIN PARTICLE

lipids
protein
enzymes
water

SERUM

Water	790 g
Carbohydrates	
lactose	46 g
glucose	70 mg
others	
Minerals	
Ca, bound	300 mg
Ca, ions	90 mg
Mg	70 mg
K	1500 mg
Na	450 mg
Cl	1100 mg
phosphate	1100 mg
sulfate	100 mg
bicarbonate	100 mg
Trace elements	
Zn	3 mg
Fe	120 µg
Cu	20 µg
many others	

Organic acids	
citrate	1600 mg
formate	40 mg
acetate	30 mg
lactate	20 mg
oxalate	20 mg
others	10 mg
Gases	
oxygen	6 mg
nitrogen	16 mg
Lipids	
glycerides	
fatty acids	20 mg
phospholipids	100 mg
cerebrosides	10 mg
sterols	70 mg
others	
Vitamins	
B vitamins	200 mg
ascorbic acid	20 mg

Proteins	
casein	+
β-lactoglobulin	3.3 g
α-lactalbumin	1.0 g
serum albumin	0.3 g
immunoglobulins	0.7 g
proteose peptone	+
others	
Nonprotein nitrogenous compounds	
peptides	+
amino acids	50 mg
urea	250 mg
ammonia	10 mg
others	300 mg
Enzymes	
acid phosphatase	
peroxidase	
many others	
Phosphoric esters	~ 300 mg
Others	.

[1] Approximate average quantities in 1 kg milk.
Note: The water in the casein micelles contains some small-molecule solutes.

gel, i.e., curd, contracts and thereby expels whey. Upon acidification of milk to pH 4.6, the casein precipitates in an almost pure form.

The serum proteins escape all these processes but are retained in ultrafiltration, as are fat globules and casein micelles. Most of the serum proteins are subject to heat denaturation (heating to, say, 80°C) and then largely precipitate onto the casein micelles.

1.2.1 Density

Mass density or volumic mass is the mass per unit of volume. It is expressed in $kg \cdot m^{-3}$ (SI units) or $g \cdot ml^{-1}$ (c.g.s. units); $1 \ g \cdot ml^{-1} = 1000 \ kg \cdot m^{-3}$. The symbol is ρ or d. The density should be distinguished from the specific gravity (s.g.), i.e., the weight of a volume of the product divided by the weight of an equal volume of water. Thus, s.g. $(T_1/T_2) = \rho \ (T_1)/\rho_{water} \ (T_2)$. Because $\rho_{water}(4°C) \approx 1 \ g \cdot ml^{-1}$, s.g. $(T_1/T_2) \approx \rho(T)$ in $g \cdot ml^{-1}$ if $T = T_1$ and $T_2 = 4°C$. For example, $\rho^{20} \approx$ s.g. (20/4). Accordingly, unlike s.g., ρ depends closely on T.

The density of a mixture of components like milk depends on the composition and can be derived from that composition according to

$$\frac{1}{\rho} = \Sigma \left(\frac{m_x}{\rho_x} \right) \tag{1.1}$$

where m_x is the mass fraction of component x, and ρ_x its apparent density in the mixture. A value of $998.2 \ kg \cdot m^{-3}$ can be taken for ρ^{20} of water, about 918 for fat, about 1400 for protein, about 1780 for lactose, and about 1850 for the residual components ($\approx$ ''ash'' $+ 0.3\%$) of milk. All of these are apparent densities in aqueous solution, not the densities of the components in a dry state (except for fat). This is because dissolution, especially that of low molar mass components, causes contraction. Furthermore, contraction is somewhat dependent on concentration.

The density of milk is rather variable. ρ^{20} of fresh whole milk is around $1030 \ kg \cdot m^{-3}$ if the fat is fully liquid. On cooling, fat solidifies (often slowly, as discussed in Sections 2.3 and 3.1); this causes a further increase in density of the milk by, for instance, about $1.2 \ kg \cdot m^{-3}$ at 10°C. Some further data are given in Table 3.4.

The density of milk increases if the solids-not-fat content increases, but it decreases if the fat content increases. From the density and the fat content, the dry matter content of milk may be approximately calculated. Taking $\rho_{fat} = 917 \ kg \cdot m^{-3}$, $\rho_{solids-not-fat} = 1622 \ kg \cdot m^{-3}$, and $\rho_{water} = 998 \ kg \cdot m^{-3}$ leads to

$$D = 1.23F + \frac{260(\rho^{20} - 998)}{\rho^{20}} \tag{1.2}$$

where D and F are dry matter and fat content of the milk (in % w/w), respectively.

1.2.2   Acidity

Acidity is usually expressed as pH. The titratable acidity of milk and milk products is the buffer capacity between the own pH and pH $\approx$ 8.3; it is usually expressed in °N = mmol NaOH $\cdot$ L^{-1} milk or milk product.

Examples of the buffering capacity (dB/d pH) and of the titration curve of milk are given in Figure 1.2. The phosphates and the proteins primarily determine the buffering. pK values for acids in milk are given in Section 2.2, for ionizable groups of protein in Table 2.14. The average contributions of milk components are approximately as follows:

2.2°N per % casein, thus on average	~5.7°N
1.4°N per % serum protein	~0.9°N
0.1°N per mM colloidal inorganic phosphate	~1.0°N
0.7°N per mM dissolved inorganic phosphate	~7.8°N
1.5–2°N for other compounds	<u>~1.7°N</u>
Total average	~17°N

The titratable acidity is specifically meant as a measure of the amount of lactic acid formed in milk (see below). But in most fresh milk the titratable acidity ranges from 14 to 21, average about 17°N. It tends to be high at the onset of lactation, say, 3°N above the later level. The pH of most samples of milk is 6.6–6.8, average 6.7 at 20°C. This implies that the H$^+$ ion activity ranges from 0.16 to 0.25 μmol $\cdot$ L^{-1}. Titratable acidity and pH exhibit a weak correlation. But when
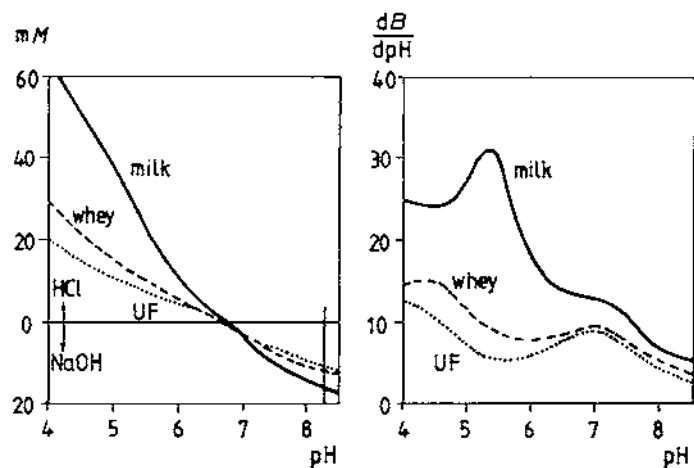
FIGURE 1.2   Titration curves and buffering capacity of milk, sweet whey, and milk ultrafiltrate, all expressed in mEq $\cdot$ L^{-1}. Approximate averages. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

acid production starts, the titratable acidity increases proportionally, whereas the pH decreases (Fig. 1.2). Lactic acid is largely dissociated (as most of the other organic acids are) until the pH falls below ~5.5. Titration down to this pH takes about equimolar quantities of lactic acid or of HCl (see also Fig. 11.4). Formation of 0.1% lactic acid (MW = 90 Da) increases the titratable acidity by $\rho^{20}/90 =$ 11.4°N. Thus, titration provides an easy method for following the amount of lactic acid formed; note that rather than the lactic acid, the repressed dissociation of acid groups and the dissociated basic groups are titrated. Essentially, the pH is a more meaningful parameter for characterizing milk acidity than titratable acidity. For example, pH determines the conformation of the protein, the activity of enzymes, and the dissociation of acids present in milk. Undissociated acids cause an acid taste and inhibit the activity of microorganisms.

The pH of milk depends closely on temperature. It is seen in Figure 1.3 that the pH of water also decreases with increasing temperature, and with that the neutral pH. The dissociation of most of the ionizable groups depends on temperature, but the dependence varies widely. Influences of heat treatment, cooling, and some other processes on pH are discussed in Section 2.2. Titratable acidity of milk somewhat decreases by heat treatment due to loss of CO_2; at very high temperature it increases due to formation of acids (Fig. 6.1).

The titratable acidity of cream is lower than that of milk because the fat globules hardly contribute. Naturally, the buffering capacity of milk serum, hence whey, is lower than that of milk, as Figure 1.2 shows. Lipolysis (enzymatic production of free fatty acids from triglycerides) causes the titratable acidity to in-
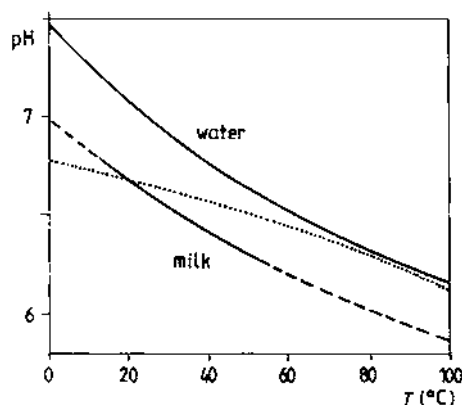
FIGURE 1.3 The pH of milk and water as a function of temperature. The dotted line refers to the pH at 20°C of milk serum obtained after ultrafiltration at the indicated temperature. Approximate values. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

crease perceptibly, especially in high-fat cream. Hydrolysis of esters (especially phosphoric esters) by enzymes would also decrease the pH.

1.2.3 Redox Potential

The oxidation–reduction or redox potential (E_h) of a redox system at 25°C is given by

$$E_h = E_0 + 0.059 \, n^{-1} \log[Ox]/[Red] \quad \text{(volts)} \tag{1.3}$$

where E_0 = standard redox potential (a characteristic of each system, dependent on temperature and especially on pH); n = number of electrons per molecule involved in the oxidation–reduction reaction; [Ox] and [Red] = molar concentrations of the compound concerned in the oxidized and reduced forms, respectively. Equation (1.3) only holds true for reversible reactions. For $n = 1$, an increase of E_h with 0.1 V thus corresponds with an increase of the relative concentration of the oxidized form from, e.g., 50% ($E_h = E_0$) to 98%.

Table 1.4 shows some redox systems occurring in milk. The standard potential mentioned is not the only determinant factor because the concentration of each redox system present also determines to what extent a system of a different standard potential can be oxidized or reduced. Moreover, the concentration determines the sensitivity of E_h to additions such as oxidants—in other words, the poising capacity.

TABLE 1.4 Standard Redox Potentials (E_0) of Some Redox Systems Important for Milk, and Their Concentration in Fresh Milk ($T = 25$°C)

Redox system	n^{1}	E_0 at pH 6.7 (V)	Concentration (μEq $\cdot$ L^{-1})
Fe^{2+}/Fe^{3+}	1	+0.77	~4[2]
Cu^{+}/Cu^{2+}	1	+0.15	<0.5
(Dehydro)ascorbate	2	+0.07	180–310[3]
Riboflavin	2	−0.20	4–14
Lactate/pyruvate	1	−0.16	([4])
Methylene blue	2	+0.02	11[5]

[1] Number of electrons transferred per molecule.
[2] Probably only partly reversible.
[3] In pasteurized milk usually less than 50% of this concentration.
[4] In fresh milk irreversible. Action and concentration depend on bacteria.
[5] Concentration in the methylene blue reduction test.

In fresh oxygen-free milk, $E_h \approx +0.05$ V, mainly dependent on ascorbate. On holding the milk, ascorbic acid shows reactions according to

L-ascorbic acid   dehydro-L-ascorbic acid   diketo-L-gulonic acid
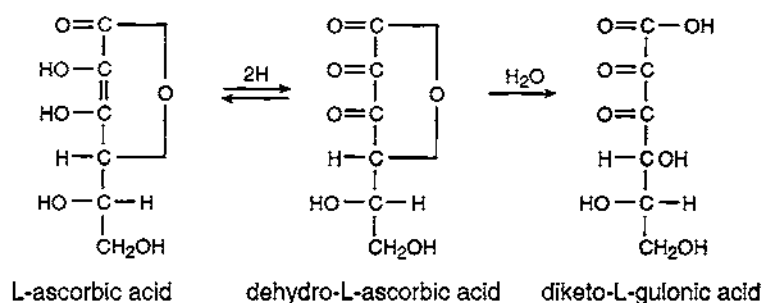
The latter reaction is irreversible, but it only occurs if both riboflavin and O_2 are present; light is active as a catalyst. Riboflavin itself is not susceptible to O_2 but is highly light-sensitive.

In actual practice, however, fresh milk always contains O_2 and, accordingly, E_h is higher, i.e., +0.2 to +0.3 V. Eventually, only dehydroascorbate is left, which subsequently can be hydrolyzed. Upon heating of milk, free sulfhydryl groups (Section 6.2) are formed; these can cause a decrease of the E_h by about 0.05 V. The cysteine-cystine system itself does not contribute to the E_h because it is not reversible at neutral pH.

Bacterial action, especially lactic acid fermentation, removes O_2 from milk and produces reductants. Accordingly, the redox potential decreases steeply, ultimately to −0.1 to −0.2 V dependent on the bacterial species. In this way, methylene blue, if added to milk, is converted to the colorless reduced form. One takes advantage of this change of color when applying the methylene blue reduction test for estimating the number of lactose-fermenting bacteria in milk. The redox potential in cheese is discussed in Section 24.8.

1.3 VARIABILITY[1]

Freshly drawn milk is not always the same. The variability in composition has been studied best, but also the structure (e.g., the size of the fat globules) varies. Furthermore, changes as mentioned in Section 1.4 may cause a certain variability. In a qualitative sense, cows' milk is fairly constant in composition.

The following are the main factors affecting composition and properties of milk.

    a.   Species, breed, and individual—in other words, genetic factors.

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[1] It may be advisable to study this section after reading Chapters 2 to 4.

    b.   Stage of lactation (this has a considerable effect; colostrum differs greatly from normal milk), as well as age of the cow, estrus, and gestation, i.e., physiological factors.

    c.   Illness of the cow, mastitis in particular.

    d.   Feed, climate, method of milking, i.e., environmental factors.

Furthermore, various extraneous components can enter milk via the cow or after milking. In this way, the variability can increase considerably. Examples are pesticides, antibiotics, and dust. In this section the natural variations in composition, structure, and properties of milk are discussed, whereas the variation caused by contamination or processing is kept aside.

    The variation in composition is not precisely known, though numerous reports on the subject have been published. This is mainly because so many factors affect the composition, and because many variations are interdependent. Furthermore, the extent of variation in the factors affecting milk composition varies greatly with climate, management practices on the farm, breeding programs, etc. In other words, results obtained in one country or region may not apply elsewhere.

1.3.1 Sources of Variation

The variability of milk thus depends on genetic factors, the physiological condition of the animal, and environmental factors.

1.3.1.1 Species

Different mammals produce milk varying widely in composition. The available data, covering about 150 species, show that dry matter content ranges from 8% to 65%, fat 0% to 53%, protein 1% to 19%, carbohydrates 0.1% to 10%, and ash 0.1% to 2.3%.

    The only species raised specifically for milk production are hoofed animals, the most important of which (cow, zebu, buffalo, goat, and sheep) are ruminants. Table 1.5 gives an impression of the variation. For example, milks of buffalo and sheep contain much fat. It is therefore common practice to dilute buffalo's milk intended for direct use with reconstituted skim milk to yield ''toned milk.'' Other differences include flavor and fat composition. Goat and sheep milk fats have low contents of butyric acid residues but high contents of caproic, caprylic, and capric acid residues (capra = goat). Buffalo's milk has comparatively large fat globules and a high colloidal phosphate content.

1.3.1.2 Breed

Often various subspecies can be distinguished within a species, but the breeds of cow are predominantly the result of selection by man. Various breeds have been obtained, according to the intended use (milk, meat, draught power) and local conditions, such as climate, feed, terrain, and customs. This has led to a

TABLE 1.5 Approximate Average Composition (% w/w) of Milk of Some Milch Animals

Animal	Genus/species	Dry matter	Fat	Casein	Serum protein	Carbohydrates	''Ash''
Donkey	*Equus asinus*	10.8	1.5	1.0	1.0	6.7	0.5
Horse	*Equus caballus*	10.8	1.7	1.3	1.2	6.0	0.5
Camel	*Camelus dromedarius*[1]	13.4	4.5	2.7	0.9	4.5	0.8
Reindeer	*Rangifer tarandus*	35	18.0	8.5	2.0	2.6	1.5
Cow	*Bos taurus*	12.7	3.9	2.6	0.6	4.6	0.7
Zebu	*Bos indicus*	13.5	4.7	2.6	0.6	4.9	0.7
Yak	*Bos grunniens*	17.7	6.7	5.5		4.6	0.9
Buffalo[2]	*Bubalus bubalis*	17.5	7.5	3.6	0.7	4.8	0.8
Goat	*Capra hircus*	13.3	4.5	3.0	0.6	4.3	0.8
Sheep	*Ovis aries*	18.8	7.5	4.6	1.0	4.6	1.0

[1] Also *Camelus bactrianus* and crossbreeds.
[2] Also called swamp or water buffalo, or carabao.

wide variability in milk yield and composition. However, the strongly directed selection over the last 100 years has decreased the variation in milk composition among typical dairy breeds. Some examples are given in Table 1.6. Variation in composition of, for instance, buffalo's or goat's milk is much wider.

1.3.1.3 Individuals

Variation in milk composition among individual cows of one breed may be greater than that among breeds (see Fig. 1.7). Differences in composition in the milk of different quarters of the udder of one cow mostly are negligible.

1.3.1.4 Stage of Lactation

This is by far the most important physiological variable. Examples are given in Figure 1.4. These refer to the average composition of the milk of 10 cows from

TABLE 1.6 Approximate Average Composition (% w/w) of the Milk of Some Breeds of Cow

Breed	Dry matter	Fat	Crude protein	Lactose	''Ash''
Friesian (in the Netherlands)	13.3	4.4	3.4	4.6	0.75
''Friesian'' (other sources[1])	12.1	3.4	3.3	4.5	0.75
Brown Swiss	12.9	4.0	3.3	4.7	0.72
Jersey	15.1	5.3	4.0	4.9	0.72

[1] For example, Holsteins in the United States and Canada.
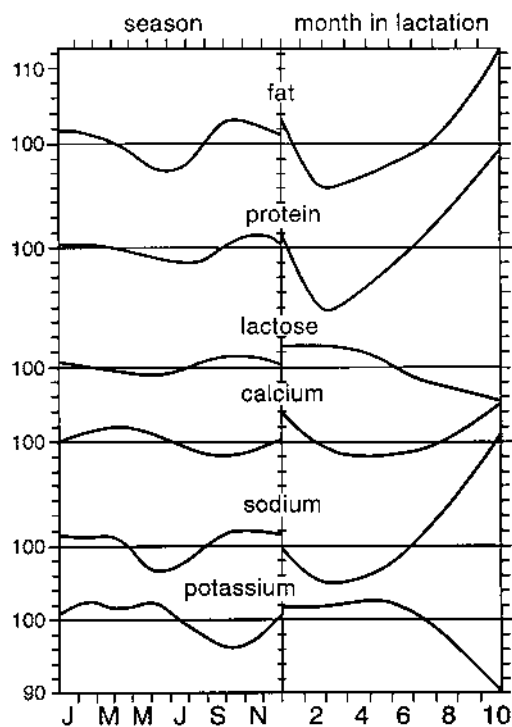
FIGURE 1.4   Content of some components in milk as a function of season and of lactation stage. The average content is put at 100%. One division corresponds to 2%. After P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

the same farm who calved in different seasons. Plotting the data as a function of season results in far smaller variations. This shows that lactation stage is the main variable, though it is difficult to separate the effect from that of other variables such as feeding regimen and grazing. Phosphate content shows a trend similar to calcium; that of chloride parallels sodium. Prolonging lactation after 10 months may eventually cause milk composition to become very different.

1.3.1.5   Colostrum

Colostrum (or beestings) has a markedly different composition. An example is given in Figure 1.5, but the compositional change varies widely among cows. In colostrum, immunoglobulins make up a large proportion of the great amount of serum proteins. Immunoglobulin content in the first colostrum is on average about
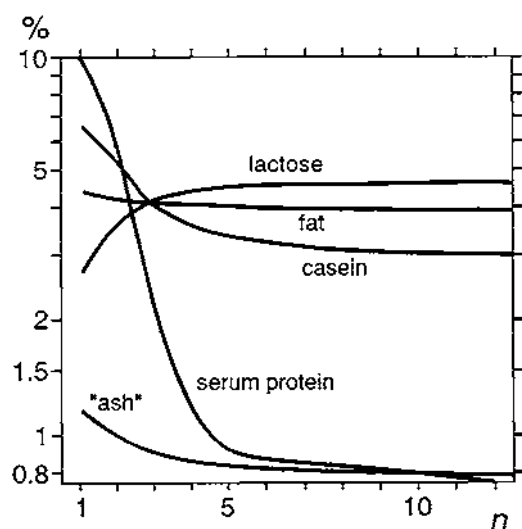
FIGURE 1.5 Example of composition (% w/w) of milk (colostrum) just after parturition. n = number of milking. Serum protein exclusive of proteose-peptone. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

7%. Colostrum gels when heated at, say, 80°C, since all of that serum protein becomes insoluble. Colostrum is also high in somatic cells, Cu and Fe. The pH can be as low as 6 and the acidity as high as 40°N.

1.3.1.6 Other Physiological Factors

Estrus and gestation do not have a great effect on milk composition, but they have on milk yield. Most of the milk components decrease slightly in concentration with age of the cow, and Na increases.

1.3.1.7 Mastitis

Severe inflammation of the udder after pathogenic bacteria have entered it causes a decrease in milk yield and a change in milk composition, and the number of somatic cells in the milk (especially polymorphonuclear leukocytes) increases. Cell count often is taken as a criterion for mastitis, though the correlation is by no means perfect. An example of milk yield and milk composition as a function of cell count is given in Figure 1.6. Presumably, the changes are smaller than shown because most determinations have been done on the first milliliters of a milking, which has a lower cell count than the average milk. Severe mastitis
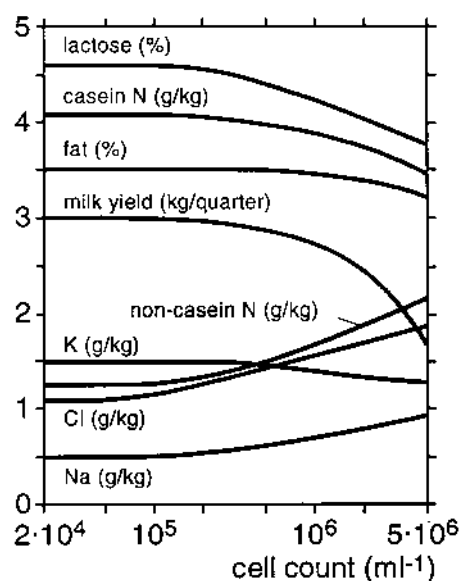
FIGURE 1.6 Examples of the concentration of some components of milk and of the milk yield as a function of somatic cell count of milk. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

causes milk composition to somewhat resemble that of blood serum, just as at the very end of lactation. Also, certain enzymes increase in activity. Usually the presence of mastitic milk does not cause significant problems for the dairy manufacturer, but mastitis causes a significant loss for the farmer.

1.3.1.8 Feed

Environmental factors may greatly affect milk yield but have less influence on milk composition. The capability of mammals to maintain a constant composition of body fluids and cells (i.e., homeostasis) is reflected in a qualitatively constant milk composition. Feed composition can affect the fat content of milk and especially its fat composition. A low-protein diet causes the protein content of the milk to decrease somewhat, whereas a high-protein diet causes nonprotein N content to increase. Several minor components are strongly affected by the content in the feed (see also Section 2.6).

1.3.1.9 Other Environmental Factors

Climate has little effect on milk composition unless it is extreme, causing heat stress. All other kinds of stress, exhaustion, and housing are associated with mostly small effects.

1.3.1.10 Milking

The shorter the time elapsed after the previous milking, the lower the milk yield and the higher the fat content will be. Hence, evening milk usually has a higher fat content than morning milk, the difference amounting to, say, 0.25% fat. During milking the fat content of milk increases (e.g., from 1% to 10%), but the difference varies markedly among cows. Incomplete milking thus can decrease the fat content of a milking, although not that of the milk on average. Short time intervals between milkings increase the susceptibility of the milk to lipolysis (Section 3.1).

1.3.1.11 Random Variations

Day-to-day fluctuations occur in the fat content especially.

1.3.2 Nature of the Variation

Most of the figures and tables of Section 1.3 give examples of variation in milk composition. Among the main components, the widest variation usually occurs in fat content. Variation in protein is less and that in lactose and ash still less. This is illustrated in Figure 1.7. The composition of a main component can also vary, especially the fatty acid pattern of the milk fat and the ratio between minerals, e.g., Na/K. Each individual protein is of constant composition except for
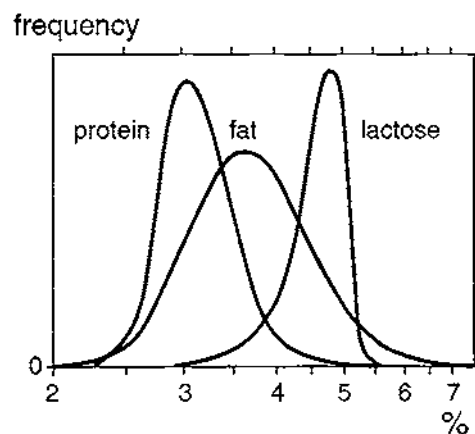
FIGURE 1.7 Frequency distributions of crude protein, fat, and lactose contents (logarithmic scale) of samples of milk from individual cows, taken throughout a year. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

genetic variants, but the ratio between them may vary somewhat; casein is relatively constant, but the proportions of the individual serum proteins are less constant, e.g., immunoglobulins and serum albumin are variable. The so-called casein number, i.e., the percentage of the N that is present in casein, largely determines the yield of cheese per kg of milk protein and thus is an important variable.

The distribution of components among the physical fractions of milk, as well as the sizes of fat globules and casein micelles are also variable. The fat globule size varies considerably, with the volume–surface average d_{vs} ranging from 2.5 to 6 μm, which corresponds to a difference by a factor of 14 in average volume. Within a single milking of one cow, fat globules and casein micelles vary in composition.

All of these variations cause variations in physical properties. Examples are density (standard deviation for individual cow samples about 2 kg · m^{-3}), titratable acidity (1°N), pH (0.04 unit), viscosity (5% relative), and refractive index of the fat (10^{-3} unit).

The extent of variation greatly depends on the sample population. The fat content of separate milkings of individual cows may range from 2% to 9%, but the range in fat content of milk as received at a dairy factory will be far smaller. Obviously, lots of milk that are each an average of a greater number of (hypothetical) sublots will generally show a smaller spread.

For the dairy manufacturer differences among different geographic regions (e.g., due to a difference in breed of cow and farming plan) and the seasonal variation are most important. The latter variation closely depends on calving pattern of the cows. In some regions (e.g., in much of California), at all times of the year an equal number of calvings occurs. This means that any seasonal pattern in milk composition cannot be due to variation in stage of lactation. Another extreme is that all cows calve within a couple of weeks at the end of winter (e.g., in much of New Zealand). This means a large variation in milk composition during the season, for by far the most part due to lactation stage; such variation can pose problems for the dairy manufacturer. In most countries, the situation is in-between, with milk production throughout the year but most cows calving somewhat before the most favorable season for milk production, be it the summer or the wet season. This would mean a moderate effect of lactation stage on seasonal variation. Figure 1.8 gives an example, and it shows that the variation is not very wide, i.e., not more than ±8% relative. Figure 1.4 also provides information.

At a decrease of the milk yield as caused by external conditions (bad weather, stress), the fat and protein contents of the milk often tend to increase slightly.

1.3.2.1 Correlations Among Variables

Correlations among variables often occur and some can qualitatively be explained. Milk is isotonic with blood, and the osmotic pressure is mainly deter-
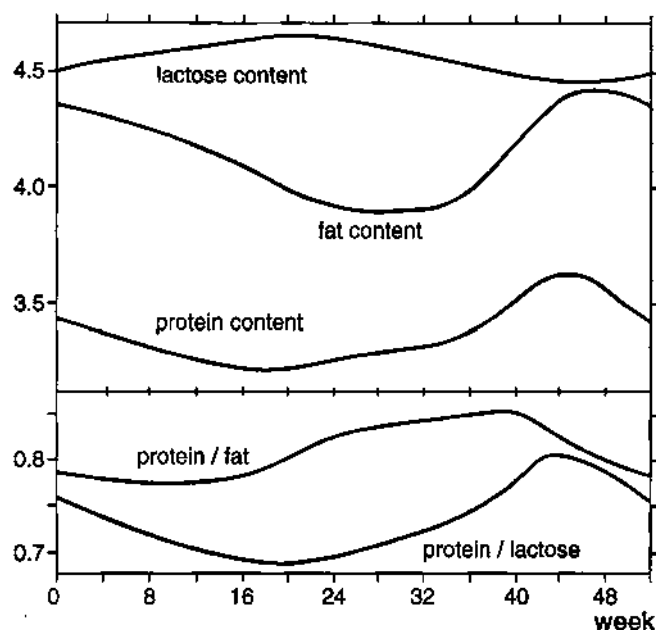
FIGURE 1.8 Examples of lactose, fat, and crude protein contents (% w/w) of milk delivered to a Dutch dairy throughout a year (January–December).

mined by lactose and the dissolved salts. Consequently, if one of these is relatively low in concentration, the other will be high. When cows suffer from mastitis or are in an advanced stage of lactation, a larger amount of low-molar mass blood components is ''leaking'' from the blood into the milk. The lactose content of the milk of these cows will be low because blood serum contains a greater amount of dissolved salts and far less sugar than milk does. Generally some mastitic cows are present, although not in large numbers, and the opposite (low content of dissolved salts) never occurs. As a result, the distribution of the lactose content of individual milkings will be negatively skewed (Figure 1.7). Similarly, the cell count frequency of quarters is positively skewed since only a few quarters suffer from mastitis; hence have a high cell count.

The negative correlation between Na and K concentrations (Figure 1.4) must be ascribed to the ''Na-K pump'' in the plasmalemma of the lactating cell. The concentrations of undissolved salts and casein are positively correlated because the casein micelles contain colloidal calcium phosphate (Section 2.2). Any part of a whole, of course, will be correlated with that whole, e.g., casein N with total N.

TABLE **1.7**   Genetic Variants of κ-casein and β-lactoglobulin, Their Frequency, and the Relation With Protein Content and Protein Composition of Milk (Samples of Milk of 10 000 Friesian Cows in the Netherlands)

Genetic variant	Frequency (%)	Crude protein (%)	Casein N/ total N	κ-casein N/ casein N
κ-A (genotype AA)	64	3.58	0.77	0.15
κ-A and B	32	3.67	0.78	0.16
κ-B	4	3.76	0.79	0.18
β-lg-A	19	3.68	0.77	0.17
β-lg-A and B	51	3.65	0.78	0.16
β-lg-B	30	3.69	0.79	0.16

The composition of milk can also be correlated with the *genetic variant* of one of the milk proteins. Table 1.7 gives examples. It shows that cows with variant B for κ-casein produce milk with a higher protein content, from which a larger part is casein, from which in turn a larger part is κ-casein. This especially holds for homozygotic animals, i.e., with genotype BB, having only variant B in their milk. To be sure, the data refer to the milk on average, since there are, of course, also other factors that affect these contents. The genetic variant of κ-casein is also correlated with other contents in the milk. For example, cows with genotype AA tend to produce milk with a higher pH and a lower calcium content. All this shows that there is not always a causal connection between genetic variant and milk composition. Presumably, the loci of the genes that encode for the differences involved (genetic variant of a protein and other composition of the milk) are close together on a chromosome, so that the genes concerned may be coupled. The genetic variant of β-lactoglobulin appears to be correlated with the protein composition of milk rather than with the protein content.

1.3.3   Some Important Variables

Following are some examples of variability in milk products caused by variation in the milk.

    a. Yields of product may vary. For example, butter yield depends on fat content of milk, cheese yield primarily on casein content, and yield of skim milk powder on solids-not-fat content.

    b. Composition of most products depends on milk composition. In standardizing cheese milk the ratio of protein to fat is essential, whereas for the composition of skim milk powder the protein/lactose ratio prevails
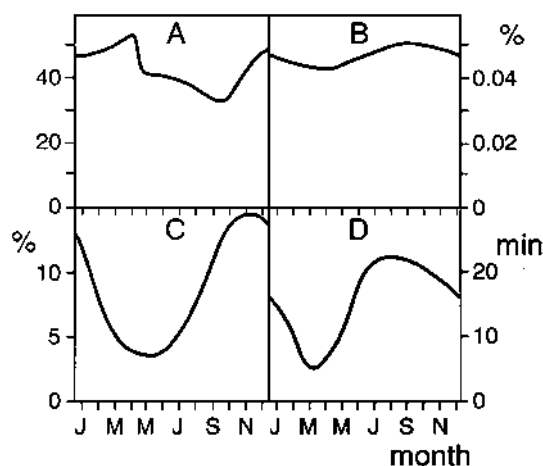
FIGURE 1.9 Examples of seasonal variation. (A) Firmness of butter (yield stress). (B) Fat content of separated milk. (C) Percentage of herd samples having a fat acidity >1.0 mEq/100 g fat. (D) Heat coagulation time of milk at 140°C. After P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

      (Fig. 1.8). The fat content of skim milk and hence of skim milk powder depends on fat globule size (Fig. 1.9B).

c.   The crystallization behavior of milk fat greatly depends on fat composition, which then affects the firmness of butter (often butter is firm in winter; Fig. 1.9A). The seasonal effect especially involves feed, although considerable differences exist among cows.

d.   Heat stability (Fig. 1.9D) is an important factor in the manufacture of evaporated milk.

e.   Fouling of heat exchangers due to deposition of protein may vary significantly with milk composition. The salt composition appears to have some effect, and this may be related to heat stability (point d). A very high content of immunoglobulins, as in colostrum, causes severe fouling. (Moreover, milk that has somewhat turned sour due to bacterial growth causes far more fouling.)

f.   Rennetability mainly is a function of Ca^{2+} activity (Section 21.3).

g.   Creaming of milk, especially rapid creaming due to the action of cold agglutinin, is highly variable. Agglutinin content of milk decreases markedly during lactation, but there are large differences among cows (see Section 3.1).

h.   Factors that inhibit or stimulate the growth of microorganisms may

vary. This concerns, for instance, the concentration of agglutinins (which probably decreases with increasing lactation stage). The concentration of Mn affects the fermentation of citric acid by some starters.

i. In terms of the flavor of milk, the ratio of the contents of dissolved salts to lactose is important. The mass ratio 100[Cl]/[lactose] varies from 1.5 to 3; in extreme cases from 1.2 to 4.5. A ratio >3 gives a salty taste. Proneness of milk to develop off-flavors also varies widely; lipase activity (Fig. 1.9C) and autoxidation rate strongly vary among cows (variation by a factor of 10 or more) and usually increase and decrease, respectively, with advancing lactation. Proneness of milk to develop ''sunlight flavor'' appears strongest in winter.

j. The color of milk, and especially that of butter and cheese, varies widely because of differences in the β-carotene content of the fat. This content closely depends on feed (grass yields yellow and hay yields white fat), but also on the ability of the cow to convert β-carotene to vitamin A. This ability varies widely among individual cows. Jersey cows give yellow-, even orange-colored milk fat, whereas buffalo, sheep, and goat give virtually colorless milk fat.

1.4   CHANGES

Milk is not a system in equilibrium. It changes, even while in the udder. This is partly because different components are formed at various sites in the mammary secretory cell and come into contact with one another after their formation. Furthermore, several changes can occur due to the milking, the subsequent lowering of the temperature, and so on. Changes may be classified as follows:

a. *Physical changes* can occur. For instance, air is beaten in during milking. Because of this, additional dissolution of oxygen and nitrogen occurs in milk. Moreover, a new structural element is formed: air bubbles. Milk contains many surface-active substances, predominantly proteins, which can become attached to the formed air–water interface. Furthermore, by contact with the air bubbles, fat globules may become damaged, i.e., lose part of their membrane.

    Fat globules may cream. Creaming is most rapid at low temperature because the globules aggregate to large flocs during the so-called cold agglutination (Section 3.1.4). On cooling, part of the milk fat starts to crystallize, the more so as the temperature is lower. But even at 0°C part of the fat remains liquid. The presence of fat crystals can strongly diminish the stability of fat globules toward (partial) coalescence.

b. *Chemical changes* may be caused by the presence of oxygen. Several substances can be oxidized. In particular, light may induce reactions,

      often leading to off-flavors. Composition of salts can vary, e.g., with temperature.

  c. *Biochemical changes.* Milk contains many enzymes that can be active. Examples are lipase, which causes lipolysis; protease, which causes proteolysis; and phosphatases, which cause hydrolysis of phosphoric acid esters.

  d. *Microbial changes* are often the most conspicuous. The best-known effect is production of lactic acid from lactose, causing an obvious decrease in pH. Numerous other changes, such as lipolysis and proteolysis, may result from microbial growth.

  e. *Processing*, of course, changes composition and properties of milk. It is intended to do so. But it often has undesirable effects. For example, high-heated milk has been markedly changed in flavor.

It may be argued that processed products should not be called milk. Still, it is common practice to do so. In this book, we will often discuss milk that has been treated in some way. Therefore, we will briefly mention the more common processing treatments.

Heat treatment is nearly always applied, and it induces numerous chemical and other changes, the extent of which depends on temperature and duration of heating. Low pasteurization (e.g., 15 s at 74°C) is a fairly mild treatment that kills most microorganisms and inactivates some enzymes but does not cause too many other changes. High pasteurization (e.g., 15 s at 90°C, but varying widely) is more intense; all vegetative microorganisms are killed, most enzymes are inactivated, part of the whey proteins become insoluble, and -SH groups become exposed. Sterilization (e.g., 20 min at 118°C) is meant to kill all microorganisms, including spores; all enzymes are inactivated; numerous chemical changes, such as browning reactions, occur; and formic acid is formed. UHT (ultrahigh-temperature) heating (e.g., at 145°C for a few seconds) is meant to sterilize milk while minimizing chemical changes; even some enzymes are not inactivated fully.

Separation, usually by means of a flow-through centrifuge called a cream separator, yields skim milk and cream. The skim milk has a very low fat content, 0.05 to 0.08%. Milk skimmed after gravity creaming has a much higher fat content. Unless stated otherwise, the term *skim milk* will refer to centrifugally separated milk. By mixing skim milk and cream, milk may be standardized to a desired fat content.

Homogenization (i.e., treatment in a high-pressure homogenizer) of milk leads to a considerable reduction in fat globule size. Such milk creams very slowly but is also altered in other respects. All sterilized milks or, more generally, all long-life liquid milk products are homogenized in practice.

Evaporation removes water, producing a more concentrated milk. Many properties are altered, e.g., the pH decreases.

Membrane processes may be applied to remove water; this is called reverse osmosis. Ultrafiltration separates milk into a concentrate and a permeate that is rather similar to milk serum. Electrodialysis removes some inorganic salts.

Fermentation or culturing of milk, usually by lactic acid bacteria, causes considerable alteration. Part of the lactose is converted to lactic acid, causing a decrease in pH to such an extent that the casein becomes insoluble. This makes the milk more viscous. The bacteria also produce other metabolites, with the kinds and concentrations depending on bacterial species.

SUGGESTED LITERATURE

- Several text and reference books exist on the topics of this chapter. The book on which we relied most is:
    P. Walstra and R. Jenness, *Dairy Chemistry and Physics*, Wiley, New York, 1984, of which a Spanish edition also exists (*Química y Física Lactológica,* Acribia, Zaragoza, 1987).
- An overview of milk composition and variability of several species is given by:
    R. Jenness, in: B.L. Larson and V.R. Smith, eds., *Lactation: A Comprehensive Treatise*, Vol. 3, Academic Press, New York, 1974, Chapter 1.
- The following book also gives much information, including composition and properties of human milk:
    R.G. Jensen, ed., *Handbook of Milk Composition*, Academic Press, San Diego, 1995.
- A monograph on factors affecting milk composition is:
    J.H. Moore and J.A.F. Rook, eds, IDF Bulletin, Document 125, International Dairy Federation, Brussels, 1980.

2

Milk Components

2.1 CARBOHYDRATES

Lactose is the major carbohydrate in milk. The sugar has been found in milks of most mammals and is unique to milk. Milk contains traces of other carbohydrates but no polysaccharides. Furthermore, glucide compounds like hexosamines and N-acetylneuraminic acid (Fig. 2.22) occur in milk, but these are largely associated with proteins and cerebrosides.

Lactose can be separated from milk by letting it crystallize. In the industrial manufacture of lactose, crystallization is used on a large scale. Lactose is, for instance, used in several foods. The pharmaceutical industry uses large amounts; almost all pills contain lactose as a filling material.

2.1.1 Chemical Properties of Lactose

Lactose is a disaccharide composed of D-glucose and D-galactose. The aldehyde group of galactose is linked to the C-4 group of glucose through a β-1, 4-glycosidic linkage (Fig. 2.1). Both sugar moieties occur predominantly in the pyranose ring form. Chemical reactions of lactose involve the hemiacetal linkage between C-1 and C-5 of the glucose moiety, the glycosidic linkage, the hydroxyl groups, and the —C—C— bond. The molecular structure of lactose has important consequences: lactose is a reducing sugar, and mutarotation occurs. That means that through the open-chain aldehyde form the aldehyde group can react and that the α and β anomers can pass from one form to another (Section 2.1.2). Suitable reagents or enzymes can cause mild oxidation of lactose, whereby the aldehyde group is converted to a carboxyl group. Somewhat more vigorous oxidation rup-

LACTOSE

(Galactose)   (Glucose)

LACTULOSE

(Galactose)   (Fructose)
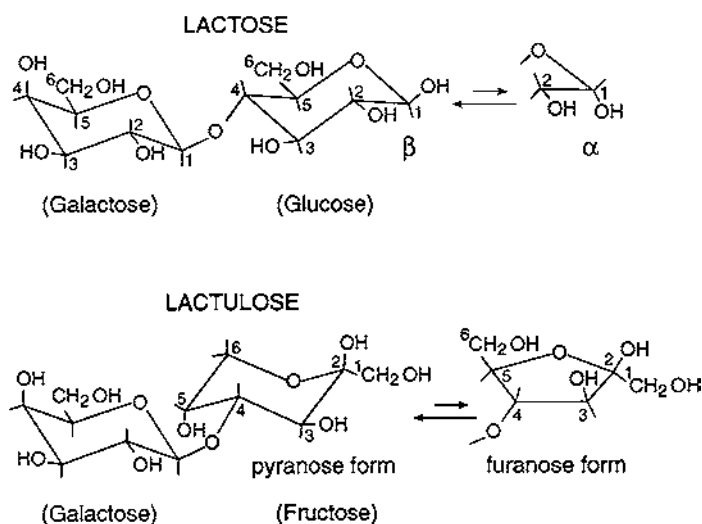
FIGURE 2.1 Chemical structure of lactose and lactulose.

tures the glycosidic linkage and produces carboxyl groups in the remaining sugars. Gentle reduction of lactose converts the aldehyde group to an alcohol group. More intense reduction cleaves the glycosidic linkage and results in the formation of alcohol groups in the remaining sugars.

Hydrolysis of lactose by acid does not occur easily. If it occurs (high temperature, low pH), many other reactions take place as well. Lactose can, however, simply be hydrolyzed using the enzyme lactase (=β-D-galactosidase). This enzyme is highly specific for the β-1,4 linkage of a galactopyranose residue. Acting on lactose, the enzyme produces, besides glucose and galactose, some di- and oligosaccharides foreign to milk, up to a few percent of the hydrolyzed lactose.

Several reactions of lactose occur when milk is heated. Lactose may isomerize into lactulose. That means that the glucose moiety converts to a fructose moiety (Fig. 2.1). Isomerization of the glucose moiety into mannose may occur as well, yielding epilactose, but the latter compound is formed in only trace amounts. In these isomerization reactions milk components are active as catalysts. (It is not fully clear as to which milk components are involved.) The quantity of lactulose in heated milk products can be used as an indicator for the intensity of the heat treatment.

On heating, caramelization also can occur. A mixture of reaction products is formed, including:

Hydroxymethylfurfural

H₂COH   COH

Furfuryl alcohol

H₂COH
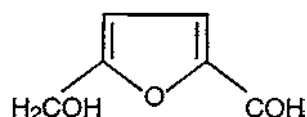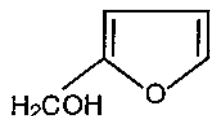
Acetol	$CH_3{\cdot}CO{\cdot}CH_2OH$
Methylglyoxal	$CH_3{\cdot}CO{\cdot}CHO$
Formaldehyde	$HCOH$
Acetic acid	$CH_3{\cdot}COOH$
Formic acid	$HCOOH$
Pyruvic acid	$CH_3{\cdot}CO{\cdot}COOH$
Levulinic acid	$CH_3{\cdot}CO{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$

The proportions of the products formed depend on concentration of sugar, pH, heating time, and temperature.

The very important Maillard reaction occurs in the presence of amino compounds (in milk it mainly concerns the ε-amino group of the lysine residue in proteins). It involves formation of a Schiff base between the amino group and the aldehyde group of lactose. The initial reaction product undergoes a series of rearrangements, yielding nitrogenous reaction products in addition to such products as mentioned above for caramelization. Further reactions lead to brown color, loss of nutritive value, and off-flavors. All these changes occur on prolonged storage, and especially during heating. The latter changes are further discussed in Section 6.2.

Lactose is approximately 0.3 times as sweet as sucrose. The sweet taste in milk is somewhat masked by the protein, primarily the casein. Because of this, whey has a sweeter taste than milk. The mixture of glucose and galactose formed by hydrolysis tastes much sweeter than lactose.

2.1.2 Physicochemical Aspects of Lactose

2.1.2.1 Mutarotation

In solution, conversion of α- to β-lactose and vice versa occurs. Thus we have

α-lactose → β-lactose, reaction constant k_1

β-lactose → α-lactose, reaction constant k_2

Both are first-order reactions. We denote the mutarotation equilibrium ratio $[\beta]/[\alpha]$ by R, and $R = k_1/k_2$. The rate of the mutarotation reaction has the constant $K = k_1 + k_2$. If we dissolve, for instance, α-lactose and if we define $x = [\alpha]/[\alpha]_{equilibrium}$, we have $\ln[R/(x - 1)] = Kt$. In other words, the proportion of the mutarotation reaction that has been completed at time t is given by $1 - e^{-Kt}$. The same holds for conversion of β-lactose. Examples are given in Figure 2.2A. These changes may be observed by using a polarimeter. The rotation of the plane of polarization then is found to change (to mutate) with time because α- and β-lactose differ in specific rotation. Hence the term ''mutarotation.'' Values for the specific rotation of α- and β-lactose are given in Table 2.1.

The mutarotation rate K depends closely on temperature (Fig. 2.2A). At 20°C and pH 6.7, $K \approx 0.37$ h^{-1}, and it increases by a factor of 3 or more per 10°C rise in temperature. At room temperature it takes many hours before mutarotation equilibrium is reached; at 70°C a few minutes. Figure 2.2B shows the great effect of pH on K. Several substances may affect the mutarotation rate. For example, the salts in milk increase the reaction rate by a factor of almost 2 as compared to the rate in water.

The mutarotation equilibrium likewise depends on temperature: $R \approx 1.64 - 0.0027T$, where T is in degrees Celsius. Thus, a change in temperature causes mutarotation.

Mutarotation depends on lactose concentration. With increasing concentration, K decreases and R changes as well. K decreases considerably if other sugars, such as sucrose, are present in high concentration. At very high lactose concentration, i.e., in amorphous lactose as, for example, occurs in spray-dried milk pow-
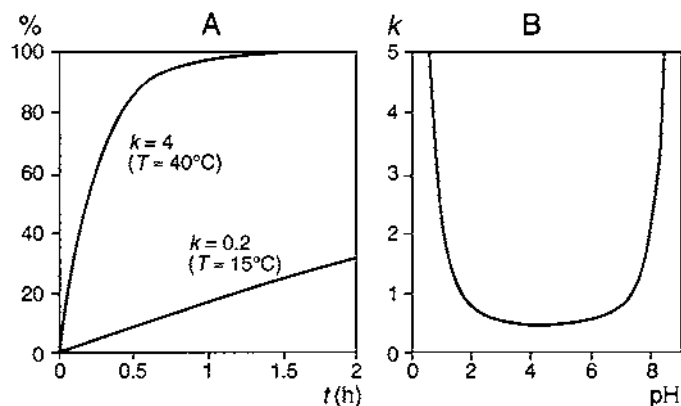
FIGURE 2.2 Mutarotation in lactose solutions. (A) Course of the reaction (% finished) against time t. (B) Mutarotation reaction constant K (h^{-1}) as a function of pH ($\sim$25°C). After H.C. Troy and P.F. Sharp, *J. Dairy Sci.* **13** (1930) 140.

TABLE 2.1 Some Properties of Lactose

A. Solubility in water (q, in g/100 g water) as a function of temperature (T, °C):

α-Lactose: log $q \approx 0.613 + 0.0128\,T$

β-Lactose: log $q \approx 1.64 + 0.003\,T$

Equilibrium solution: $q = 12.48 + 0.2807T + 5.067 \times 10^{-3}T^2 + 4.168 \times 10^{-6}T^3 + 1.147 \times 10^{-6}T^4$.

Attention: $T \leq 93.5°C$

B. Density, viscosity, and refractive index as a function of concentration

Concentration (g lactose/ 100 g water)	Density of solution at 20°C (kg · m⁻³)	Apparent density of lactose dissolved in water (kg · m⁻³)	Viscosity of solution (mPa · s)		Refractive index of solution at 25°C, λ = 589 nm
			20°C	60°C	
0	998.2	—	1.00	0.47	1.3325
10	1043	1750	1.38	0.70	1.3484
20	1082	1629	2.04	0.90	1.3659
30	1124	1592	3.42	1.29	
40	1173	1591	7.01	2.19	

C. Specific rotation α:

α is expressed in degrees of arc of rotation per cm pathlength in a hypothetical solution of 1 g anhydrous lactose per ml lactose solution. The rotation depends on temperature (T, °C), on the wavelength of the light (λ), and somewhat on concentration (C, g/100 ml solution), etc.

For equilibrium solutions:

$\alpha_D = 56.75 - 0.0017C - 0.058T$ (D line, λ = 589 nm)

$\alpha_{Hg} = 66.25 - 0.007C - 0.054T$ (Hg line, λ = 546 nm)

For pure α-lactose: $\alpha_D = +91.1$ at 20°C

For pure β-lactose: $\alpha_D = +33.2$ at 20°C

der, after equilibration $R \approx 1.25$, independent of temperature; mutarotation may still occur, but extremely slowly.

2.1.2.2 Solubility

As seen in Figure 2.3, α- and β-lactose differ considerably in solubility and in the temperature dependence of solubility. If α-lactose is brought in water, much less dissolves at the outset than later. This is because of mutarotation: α-lactose is converted to β, hence the α-concentration diminishes and more α can dissolve. If β-lactose is brought in water, more dissolves at the outset than later (at least below 70°C): on mutarotation more α-lactose forms than can stay dissolved, and α-lactose starts to crystallize.

The solubility thus depends partly on the mutarotation equilibrium, the rate of dissolution on the mutarotation rate. The so-called final solubility is identical whether we dissolve α- or β-lactose. It is $R + 1$ times the initial solubility of α-lactose. This applies below 93.5°C because above this temperature β-lactose determines the final solubility. At low temperatures, it takes a long time to reach equilibrium.

Lactose solutions can be supersaturated easily and to a considerable extent.
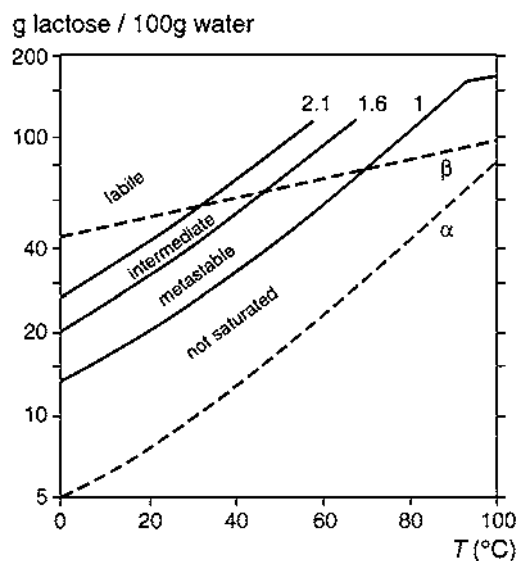
FIGURE 2.3 Solubilities of α- and β-lactose, final solubility of lactose (curve 1), and supersaturation by a factor of 1.6 and 2.1 as a function of temperature. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

This is indicated roughly in Figure 2.3. At concentrations over 2.1 times the saturation concentration, spontaneous crystallization occurs rapidly, probably because of homogeneous nucleation (i.e., formation of nuclei in a pure liquid). At less than 1.6 times the saturation concentration, seeding with crystals usually is needed to induce crystallization, unless we wait a very long time; the solution is thus metastable. In the intermediate region, crystallization depends on several factors, such as time.

2.1.2.3 Crystal Forms

Usually, α-lactose crystallizes as a hydrate containing one molecule water of crystallization. The crystals are very hard, slightly hygroscopic, often fairly large, and dissolve slowly. The water of crystallization is very strongly bound. Above 93.5°C, anhydrous β-lactose crystallizes from an aqueous solution. β-Lactose is not very hygroscopic, and it dissolves quickly; its solubility is good. Obviously, dehydrating α-hydrate is difficult. It may cause problems when determining the dry matter content of milk and milk products; this determination implies evaporation of water at elevated temperature. Maintaining the temperature >93.5°C during the assay is paramount to prevent formation of α-lactose hydrate crystals.

Amorphous lactose is formed during rapid drying, as in a spray drier. It is present in the glassy state, which means that many properties, including hardness, density, and specific heat, are similar to those of the crystalline sugar but that the packing of the molecules does not show perfect order. Amorphous lactose contains at least a few percent of water and can quickly dissolve on addition of water. But then, α-lactose hydrate may start to crystallize. If the water content of the amorphous lactose is low, say 5%, crystallization is postponed. However, the product attracts water from moist air, and when moisture content rises to about 8%, α-lactose hydrate starts to crystallize (at room temperature). The postponed crystallization is an important factor in relation to spray-dried powders made from skim milk or whey because it leads to hard lumps in the powder; eventually, the whole mass of powder turns into one solid cake.

Several other crystal modifications of lactose may occur. Figure 2.4 gives a survey, including the transitions. In principle, it gives the methods for preparing the different modifications. In practice, it is almost impossible to obtain pure crystals of whatever form. For example, α-hydrate usually contains a few percent of β-lactose, and vice versa.

The different forms mentioned are different crystal modifications, i.e., they have different crystal lattices. As a result, the properties are different. For instance, the stable anhydrous α modification, also called S-lactose, is quite soluble in water. But a concentrated solution of it is unstable, which means that α-hydrate soon starts to crystallize. S-Lactose is not very hygroscopic. The unstable anhydrous α form, however, is hygroscopic; accordingly, its transition to α-hydrate occurs easily, and the sugar dissolves faster, though not better, than α-hydrate.
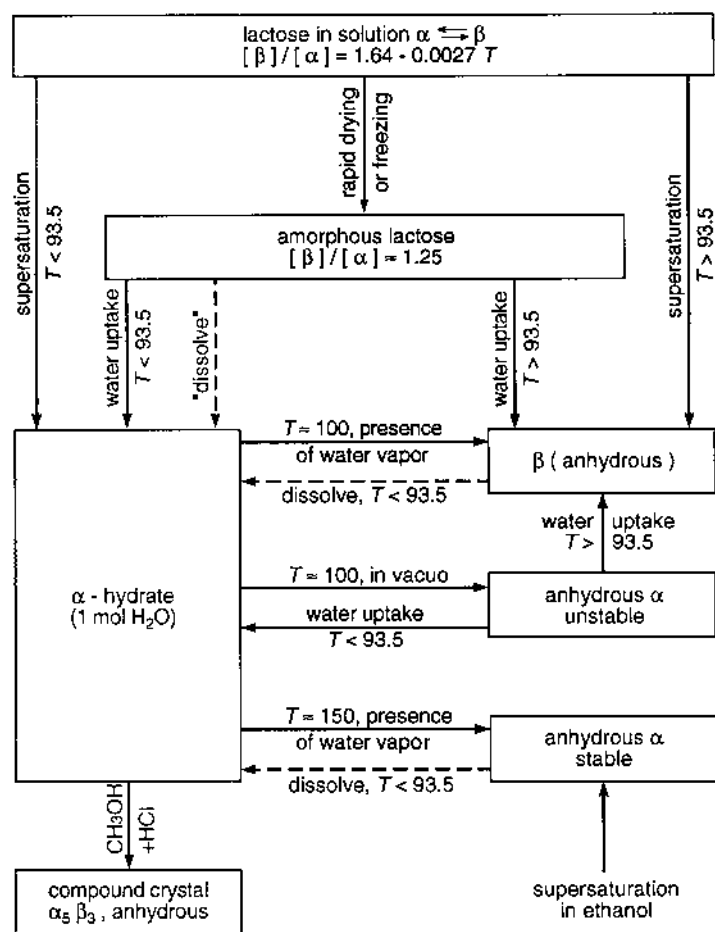
FIGURE 2.4 The different forms of lactose. T = temperature (°C).

2.1.2.4 Crystallization of α-Lactose Hydrate

This crystallization is of great practical importance. Because α-hydrate is poorly soluble, it may crystallize in some milk products, especially ice cream and sweetened condensed milk. Large crystals can easily be formed because both nucleation and crystal growth are slow. We usually have to add numerous tiny seed crystals to ensure the rapid formation of sufficient, hence small-sized, crystals. To prevent segregation and development of ''sandiness'' in milk products, the largest crystals formed should be no more than 10 μm in size. This implies that at least 10^{10} crystals per gram of crystalline lactose should be present.

α-Hydrate crystals can have many geometrical forms (but the crystal lattice is always the same). The commonest shape is the ''tomahawk,'' depicted in Figure 2.5. Usually, the crystal does not grow in the direction of the b axis, i.e., the crystal faces $0\bar{1}0$ and K or $1\bar{5}0$. Likewise, the $0\bar{1}1$ lateral faces do not grow at all. Consequently, the ''apex'' of the crystal is also the point where the crystal started to grow. Furthermore, crystal growth is slow, far slower than may be accounted for by the combined effects of mutarotation and diffusion of α-lactose to the crystal.

Presumably, there is some difficulty of fitting molecules into the crystal lattice. But otherwise the observations of the preceding paragraph are largely explained by inhibition by β-lactose. It appears as if β-lactose fits well into the $0\bar{1}0$ and $0\bar{1}1$ faces of the crystal lattice but then prevents any further uptake of α-lactose. Growth of other faces is inhibited as well (Fig. 2.6 and Table 2.2). If very little β-lactose is present (this condition is difficult to accomplish), the $0\bar{1}1$ faces grow fast, causing formation of needle crystals. Several other substances can retard crystal growth; the individual crystal faces are inhibited in different ways, which leads to variation in crystal habit. Examples of growth ''inhibitors'' are given in Table 2.2. Some inhibitors, such as riboflavin, are present in milk. Some additions can speed up the growth rate of certain crystal faces.

One particular crystal growth inhibitor should be mentioned. If α-lactose hydrate is purified by recrystallization, its rate of crystal growth decreases (Table 2.2). Moreover, the pH of the lactose solutions falls upon further recrystallization.
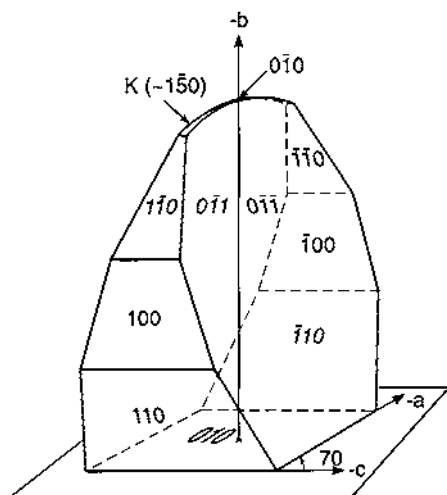
FIGURE 2.5 Common shape of the α-lactose hydrate crystal. The main axes (a, b, c) and the indices of the various faces are given.
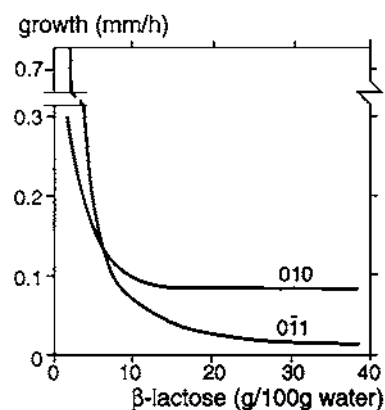
FIGURE 2.6 Effect of the concentration of β-lactose on the growth rate of some faces of the α-lactose hydrate crystal. Supersaturation of α-lactose is by 170%. After A. van Kreveld, *Neth. Milk Dairy J.* **23** (1969) 258.

It appears that a crystal growth inhibitor is present; it has a stronger affinity for the lactose crystal than α-lactose itself. The inhibitor is a mixture of lactose monophosphates; its concentration in milk is about 15 mg · L^{-1}. It particularly inhibits at low supersaturation and causes inhibition of nucleation in lactose solutions. The substance can be removed by ion exchange.

TABLE 2.2 Examples of the Rate of Growth of Some Faces of an α-Lactose Hydrate Crystal as Affected by Liquid Composition

Supersaturation (%)	Remarks	Growth (μm · h^{-1}) of face			
		010	110	100	1$\bar{1}$0
55	—	3.8	3.3	1.3	0.3
55	+ 10 ppm gelatin	1.2	1.0	1.0	0.4
55	+ 100 ppm riboflavin	2.7	0.0	0.0	0.0
55	+ 10 ppm TMODAC[1]	0.0	0.5	0.6	0.0
120	—	43	34	21	12
55	Own pH (~4)	3.2	2.7	1.6	0.4
55	pH 7	6.6	5.0	2.7	1.2
55	3 × recrystallized	0.2	0.7	1.3	0.5
55	Nonionic[2]	19.1	9.1	3.1	1.2
55	Nonionic + inhibitor[3]	0.0	0.0	0.9	0.5

[1]Trimethyloctadecylammonium chloride.
[2] Solution passed through an anion exchanger.
[3] Lactose monophosphates.

2.1.3   Lactic Acid Fermentation

Lactose is the main source of energy for most of the bacteria growing in milk. Commonly, the organisms attack lactose by hydrolyzing it to form galactose and glucose. The latter molecules are each fermented into lactic acid ($CH_3 \cdot CHOH \cdot CO_2H$), but part of the galactose may not be metabolized. Some lactic acid bacteria, classified as homofermentative, produce only lactic acid according to

$$Glucose + 2ADP + 2H_3PO_4 \rightarrow 2\ Lactate + 2ATP + 2H_2O$$
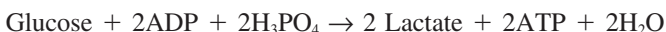
Other bacteria, classified as heterofermentative, produce lactic acid as well as CO_2, acetic acid, and ethanol. This subject is discussed further in Section 11.1.

Accordingly, lactic acid is an essential component of many milk products. Most bacteria produce about 1% lactic acid in milk, but some of these can reach as much as 2%. Such high lactic acid concentrations inhibit growth of most microorganisms.

2.2   SALTS

Milk contains inorganic and organic salts. The concept of ''salts'' thus is not equivalent to ''mineral substances.'' Salts are by no means equivalent to ''ash'' because ashing of milk causes loss of organic acids including citrate and acetate, and because organic phosphorus and sulfur are transferred to inorganic salts during ashing. Mass concentrations of salts can be expressed in various ways, e.g., as element (e.g., P), acid residue (PO_4^{3-}), or oxide (P_2O_5).

2.2.1   Composition and Distribution Among the Phases

Average concentrations of salts in milk are given in Tables 1.3, 2.3, and 2.6. The salt composition varies, but the various components do not vary independently of each other.

Not all of the salts are dissolved, and not all of the dissolved salts are ionized. The casein micelles contain the undissolved salts. In addition to counterions of the negatively charged casein, the micelles contain the so-called colloidal calcium phosphate, which also contains Mg, citrate, Na, and K, as well as presumably small quantities of other ions. The colloidal phosphate is amorphous, can vary in composition, and may have ion exchange properties.

The distribution of phosphorus among the fractions is even more intricate. Details are given in Table 2.4. It is important to note that phosphatases present in milk may hydrolyze phosphoric esters, causing the content of organic (esterified) phosphate to decrease and that of inorganic phosphate to increase. Milk contains sulfur, again in several forms. Not more than 10% of the sulfur in milk, amounting

TABLE 2.3   The Most Important Salts in Milk and Their Distribution
Between Serum and Casein Micelles

Compound	Molar mass (Da)	Range (mmol/kg)	Average (mg/100 g)	Fraction present in serum	In micelles (mmol/g dry casein)
Cations					
Na	23	17–28	48	0.95	0.04
K	39.1	31–43	143	0.94	0.08
Ca	40.1	26–32	117	0.32	0.77
Mg	24.3	4–6	11	0.66	0.06
Amines		~1.3		~1	
Anions					
Cl	35.5	22–34	110	1	
CO_3	60	~2	10	~1?	
SO_4	96.1	~1	10	1	
PO_4[1]	95	19–23	203	0.53	0.39
Citrate	189	7–11	175	0.92	0.03
Carboxylic acids		1–4		~1?	
Phosphoric esters[2]		2–4		1	

[1] Inorganic only.
[2] Soluble.

to about 36 mg/100 g milk, is present as inorganic sulfate, whereas the remainder
is present in protein.

The dissolved salts affect various milk properties, e.g., protein stability.
These salts are only present in the serum. Note that the solute content in the
serum approximates 1.09 times the content in milk; in the plasma it approximates
1.04 times the content in milk (see also Section 9.1).

The composition of the salt solution of milk is best determined from a
dialyzate of milk. The solution can be obtained by dialysis of water against a
large excess of milk, as it is in equilibrium with the colloidal particles and dis-
solved proteins in milk. It does not reflect precisely the concentrations of the
various dissolved salts in the water. To begin with, part of the water in milk is
not available as a solvent (Section 9.1). Second, the proteins have a ''diffuse
double layer,'' so that they are accompanied by counterions. Of the cations asso-
ciated with the casein micelles (Table 2.3), all of the Na and K, most of the Mg,
and a far smaller portion of the Ca are present as counterions. The serum proteins
also take along some counterions.

Trace elements are elements of which not more than a trace is found in
milk. Of these, zinc is present in the highest concentration, i.e., approximately
3 mg/kg of milk, while the other elements are present in far lower concentrations.

TABLE 2.4 Approximate Distribution of Phosphorus in Milk

Type	Location	Distribution (%)	Dialyzable against water	Dialyzable at low pH	Soluble in TCA[1]	Extractable with ethanol/ether
Esterified to casein	Casein micelles[2]	22	No	No	No	No
Esterified in phospholipids	Fat globules and serum	1	No	No	No	Yes
Various esters	Serum	9	Yes	Yes	Yes	Yes
Inorganic, "colloidal"	Casein micelles	32	No[3]	Yes	Yes	No
Inorganic, dissolved	Serum	36	Yes	Yes	Yes	No

[1] Final concentration 12% trichloroacetic acid.
[2] A small part in the serum, especially at low temperature.
[3] Partly dialyzable against an excess of water.
Note: Milk contains approximately 1 g P per kg.

TABLE 2.5   Trace Elements in Cows' Milk ($\mu g \cdot L^{-1}$)

Element	Symbol	Contents reported[1]	Average content
Aluminum	Al	50–2100	500
Arsenic	As	10–400	50?
Barium	Ba	tr–110	tr
Boron	B	30–800	200
Bromine	Br	20–25 000	600?
Cadmium	Cd	0.02–78	0.03
Cesium	Cs	3–46	?
Chromium	Cr	5–82	15
Cobalt	Co	0–20	1
Copper	Cu	10–1200	25
Fluorine	F	20–700	180
Iodine	I	5–700	50
Iron	Fe	100–2400	200
Lead	Pb	1–65[2]	2.3
Lithium	Li	tr–29	tr
Manganese	Mn	3–370	20
Mercury	Hg	<0.1	tr
Molybdenum	Mo	5–150	70
Nickel	Ni	0–180	30?
Rubidium	Rb	100–3400	2000?
Selenium	Se	4–1200	40?
Silicon	Si	1300–7000	1400
Silver	Ag	tr–54	?
Strontium	Sr	45–2000	170?
Tin	Sn	0–1000[3]	tr
Titanium	Ti	2–500	?
Vanadium	V	tr–310	0.1?
Zinc	Zn	220–19 000	3900

[1] Contents reported in literature vary widely, caused by contamination of the milk involved and inaccuracies of determinations.
[2] In milk packed in cans up to 600 $\mu g \cdot L^{-1}$.
[3] In milk packed in cans up to 200 mg $\cdot L^{-1}$.

The number of known trace metals increases with the development of increasingly sensitive methods of analysis. Trace elements are listed in Table 2.5.

Trace elements are natural components in milk. Concentrations of some of these elements in milk can be increased by increasing their level in the feeding ration of the cow. Consequently, their concentration in milk can vary widely. For instance, Se can range from 4 to 1200 µg/kg of milk. Concentrations of other metals are not affected by cattle feed, except on shortage (e.g., Cu, Fe), or if

extreme levels in the ration cause poisoning of the cow (e.g., Pb). Finally, some elements can enter the milk by contamination following milking. Contamination can considerably increase the concentrations. For instance, the natural Cu content of milk is about 20 $\mu g \cdot kg^{-1}$ (colostrum contains more); contact of milk with bronze parts in milking utensils or with copper pipes can increase its Cu content easily to 1 $mg \cdot kg^{-1}$. Fe also can readily enter the milk due to contamination.

Little is known about the distribution of the trace elements among the fractions. Part of the elements are likely to be associated with protein, e.g., some heavy metals with lactoferrin, whereas most of the other elements are dissolved. About 10% of Cu and nearly half of Fe is associated with the fat globule membrane. Zn is predominantly in the colloidal phosphate.

Some of the trace elements are important from a nutritional point of view, whereas other elements (e.g., Cd, Hg, and Pb) are toxic, though they are hardly ever detected in milk in too high a concentration. For the dairy manufacturer, Cu is of great importance due to its catalytic action on fat autoxidation. ''Natural Cu'' in milk does not promote oxidation, or does so hardly at all, but ''added Cu'' often does, even when added in minute amounts. Mn is of importance in the metabolism of some lactic acid bacteria, especially for fermentation of citrate, and in some milks the Mn content is too low for production of diacetyl by leuconostocs.

2.2.2 Properties of the Salt Solution

Only the dissolved salts are considered here, i.e., roughly the salts in the milk serum. The composition does not follow simply from Table 2.3, mainly because of the extensive association of ions. Some of the acids and bases in milk (phosphoric acid, carbonic acid, secondary amines, etc.) are not fully ionized at milk pH. But some of the salts may also be partly associated. This primarily concerns binding of Ca^{2+} and Mg^{2+} to $citrate^{3-}$ and to HPO_4^{2-}. Consequently, the concentration of Ca^{2+} ions is much lower than that of dissolved Ca, because $Ca\text{-}citrate^-$ and $CaHPO_4$ are present in appreciable amounts. Also other salts, e.g., the chlorides of Na, K, Ca, and Mg, are not completely ionized. The approximate ion composition can be calculated from the atomic composition of the milk salt solution and the association constants. Results are given in Table 2.6.

Such calculations should be based on activities (a, in mol/L) rather than on concentrations (m, in mol/L). Activities govern reaction rates and thereby the state of ionization. By definition, the relation between a and m of a substance x is given by

$$a_x \equiv \gamma_x m_x \tag{2.1}$$

where γ is the activity coefficient. If the total ionic strength (I) is not too high,

TABLE 2.6 Estimated Average Composition of Milk Serum, i.e., the Salt Solution of Milk, Including the Other Dissolved Substances[1]

Cations					Anions					Neutral	
Ion	m	mz	mz^2	a	Ion	m	$-mz$	mz^2	a	Molecule	m
K^+	36.4	36.4	36.4	29	Cl^-	30.7	30.7	30.7	24.6	KCl	0.7
Na^+	21.1	21.1	21.1	17	SO_4^{2-}	0.9	1.8	3.6	0.4	NaCl	0.4
Ca^{2+}	2.1	4.2	8.4	0.85	KSO_4^-	0.1	0.1	0.1	0.1	$CaSO_4$	0.1
$CaCl^+$	0.3	0.3	0.3	0.2	$Citrate^{3-}$	0.3	0.9	2.7	—	$CaHPO_4$	0.5
$CaH_2PO_4^+$	0.1	0.1	0.1	0.1	$HCitrate^{2-}$	0.1	0.2	0.4	—	$MgHPO_4$	0.3
Mg^{2+}	0.8	1.6	3.2	0.35	$CaCitrate^-$	7.0	7.0	7.0	5.6	KH_2PO_4	0.2
$MgCl^+$	0.1	0.1	0.1	0.1	$MgCitrate^-$	2.0	2.0	2.0	0.6	NaH_2PO_4	0.1
RNH_3^+	1.3	1.3	1.3	1.0	$RCOO^-$	2.5	2.5	2.5	2.0	$CaROPO_3$	0.2
H^+				2×10^{-4}	HPO_4^{2-}	3.0	6.0	12.0	1.3	CO_2	0.6
					$H_2PO_4^-$	7.3	7.3	7.3	5.8	Lactose	147
					$KHPO_4^-$	0.5	0.5	0.5	0.4	Urea	5
					$NaHPO_4^-$	0.4	0.4	0.4	0.3	Other	4
					$ROPO_3^{2-}$	1.7	3.4	6.8	0.7		
					$HROPO_3^-$	0.6	0.6	0.6	0.5		
					$KROPO_3^-$	0.2	0.2	0.2	0.1		
					HCO_3^-	1.4	1.4	1.4	1.1		
Total	62.2	65.1	69.8		Total	58.7	65.0	78.2		Total	159.1

[1] pH = 6.7; m = concentration in mmol/L solution; z = valency; a = free ion activity. To convert the composition to millimoles per kg of milk, multiply by ~0.904; to convert to millimoles per kg water in milk, multiply by ~1.045.

say, $I < 0.1$, the free-ion activity coefficient of ionic species in water of room temperature is given by

$$\gamma \approx \exp\left(-0.8z^2 I^{1/2}\right) \tag{2.2}$$

where z is the valency of the ion (i) involved. I is defined by

$$I = \frac{1}{2} \sum m_i z_i^2 \tag{2.3}$$

Results in Table 2.6 show that in milk $I \approx 0.075$. Hence, γ for mono-, di-, and trivalent ions in milk would be 0.80, 0.42, and 0.14, respectively. These are approximate values. All association and dissociation constants and solubility products as listed in handbooks and the like are so-called intrinsic constants that refer to activities. They only apply to concentrations if $I \to 0$, where $\gamma \to 1$ and, hence, $a \to m$. Relating association constants to concentrations yields ''stoichiometric'' constants; in these cases, the ionic strength always should be recorded.

Neglecting the association of ions and not allowing for the activity coefficients can cause considerable deviations, especially with respect to polyvalent ions. Let us consider, for example, the solubility of calcium citrate. The solubility product of $Ca_3citrate_2$ is given as 2.3×10^{-18} mol$^5 \cdot$ kg^{-5} (Table 2.7). If one takes the total concentrations of calcium and citrate in milk serum (these amount to, say, 10 mmol $\cdot$ kg^{-1}), a concentration product of 10^{-10} mol$^5 \cdot$ kg^{-5} is found, i.e., about 4×10^7 times the solubility product. In other words, milk serum may be supersaturated with respect to calcium citrate by a factor of $(4 \times 10^7)^{1/5} = 33$. But, obviously, only the Ca^{2+} and citrate^{3-} ions must be considered. From Table 2.6 we see that

$$[Ca^{2+}]^3 \times [citrate^{3-}]^2 = (2.1 \times 10^{-3})^3 \times (0.3 \times 10^{-3})^2 \approx 8.3 \times 10^{-16}$$

This corresponds to a supersaturation by a factor of $360^{1/5} \approx 3.2$ (320%). The solubility product is, however, an intrinsic property. Consequently, the activities rather than the concentrations must be inserted, and the product of the ions be

TABLE 2.7 Intrinsic Solubility Products at 20°C (Ion Activities in moles/kg Water)

Compound	Solubility product	Unit
$Ca_3Citrate_2$	2.3×10^{-18}	mol$^5 \cdot$ kg^{-5}
$CaHPO_4 \cdot 2H_2O$	2.6×10^{-7}	mol$^2 \cdot$ kg^{-2}
$Ca_4H(PO_4)_3$	1.2×10^{-47}	mol$^8 \cdot$ kg^{-8}
$Ca_3(PO_4)_2$	$\sim 2 \times 10^{-29}$	mol$^5 \cdot$ kg^{-5}
$Ca_5OH(PO_4)_3$	$\sim 10^{-58}$	mol$^9 \cdot$ kg^{-9}
$Ca(CH_3CHOHCO_2)_2$	$\sim 10^{-4}$	mol$^3 \cdot$ kg^{-3}
$MgHPO_4 \cdot 3H_2O$	1.5×10^{-6}	mol$^2 \cdot$ kg^{-2}

multiplied by $(\gamma_{Ca^{2+}})^3 \times (\gamma_{citrate^{3-}})^2 \approx 0.42^3 \times 0.14^2 \approx 1.5 \times 10^{-3}$. Hence, the product of activities is only about half the solubility product, i.e., the concentration is 88% of the saturation concentration. Incidentally, the data used are not very precise, so the result may not be exactly correct. Milk must be about saturated with respect to calcium citrate because citrate is partly undissolved (Table 2.3).

Addition of a neutral salt, e.g., NaCl, to milk will cause some further association of Na^+ with the citrate^{3-} and of Cl^- with the Ca^{2+}. More importantly, the ionic strength increases, as a result of which all ion activity coefficients decrease [see Eq. (2.2)] and, consequently, the ion activities and the activity product decrease as well. As a result, more calcium citrate can dissolve. (For example, increasing the ionic strength from 0.08 M to 0.12 M would increase the solubility of Ca$_3$citrate$_2$ by about 35%.) The general rule thus is that any increase of the ionic strength increases the solubility of salts.

Increasing the ionic strength also causes an increase of the dissociation of salts. Consider the equilibrium

$$Ca^{2+} + HPO_4^{2-} \leftrightarrows CaHPO_4$$
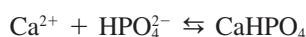
The dissociation constant is

$$K_D = a_{Ca^{2+}} \times a_{HPO_4^{2-}} / a_{CaHPO_4}$$

We may state that $a_{CaHPO_4} = [CaHPO_4]$, because in diluted mixtures the activity coefficient of electroneutral molecules is about 1. Increasing I will reduce the activity coefficients of Ca^{2+} and HPO_4^{2-}. Since K_D is constant, $[Ca^{2+}]$ and $[HPO_4^{2-}]$ will increase, whereas $[CaHPO_4]$ will decrease. In other words, the dissociation increases.

The same holds for acids in milk. The stoichiometric pK of monovalent acids is shifted downward by about 0.1 pH unit in milk, as compared to $I \rightarrow 0$. The stoichiometric pK is defined by

$$\log([ion]/[acid]) = pH - pK \tag{2.4}$$

pK values for acids in milk are approximately as follows:

Phosphoric acid	2.1,	7.1,	12.4
Citric acid	3.0,	4.5,	4.9
Carbonic acid	6.3,	10.0	
Fatty acids	4.7		

Note that the pH is an intrinsic quantity: $pH = -\log a_{H^+}$.

2.2.2.1 Ionic Strength

The total ionic strength I in milk serum is thus estimated to be 0.075 M. The ionic strength in milk affects the thickness of the diffuse double layer $1/\kappa$ around

negatively charged colloidal particles; the Debye length $1/\kappa$ is a parameter in the theory of the stability of lyophobic colloid particles. The electrical charge of a particle such as a casein micelle is screened by counterions, and the electrostatic potential at a distance h from the particle is proportional to $\exp(-h\kappa)$ times the charge potential at the surface. Assuming the relative dielectric constant of milk serum to be 80, we have $1/\kappa = 1.1$ nm. Consequently, electrostatic interactions in milk will usually be of little importance over distances greater than, say, 2.5 nm. Divalent counterions (Ca^{2+}, Mg^{2+}) are roughly 60 times as active as Na^+ and K^+ in the flocculation of hydrophobic colloid particles. Though present in small amounts, Ca^{2+} and Mg^{2+} are thus of great importance. Ionized calcium has a large effect on the properties of casein and casein micelles. Consequently, the calcium ion activity ($a_{Ca^{2+}}$, Section 2.2.4.4) is an essential parameter for milk and milk products. Variation in citrate concentration mainly determines the natural variation in $a_{Ca^{2+}}$.

The electric conductivity of milk is about 0.5 A $\cdot$ V$^{-1}\cdot$m^{-1} (variation $\sim$ 0.4–0.55) at 25°C. This roughly corresponds to the conductivity of 0.25% (w/w) NaCl in water.

2.2.2.2 Colligative Properties

Freezing point depression, osmotic pressure, (1 $-$ water activity), etc., are called the colligative properties. Nonionic solutes as well as ions determine the magnitude of these properties. A freezing point depression of 0.53 K (which corresponds to that of a solution of 0.85% of NaCl) is calculated from the total concentration of dissolved substances (0.28 mol/L solution), the molar freezing point depression of water (1.86 K for 1 mol in 1 kg of water), the average osmotic coefficient ($\sim$0.93 for ionic species and 1.00 for neutral molecules), and from the fact that 1 L of milk serum contains about 950 g of water. The calculated value corresponds satisfactorily to the observed freezing point depression of about 0.53 K, which varies among milk samples from $\sim$0.515 to $\sim$0.55 K.

The freezing point depression of milk is very constant (relative standard deviation between individual milkings about 1%) inasmuch as it is proportional to its osmotic pressure, which is essentially equal to that of blood, which in turn is kept almost constant. The osmotic pressure of milk is about 700 kPa (7 bar) at 20°C. It follows from Table 2.6 that lactose accounts for over half of that value, complemented by K^+, Na^+, and Cl^-. Furthermore, a boiling point elevation of milk of 0.15 K is calculated from the molar concentration, and a water activity of 0.993.

2.2.3 Colloidal Calcium Phosphate

Table 2.3 shows that part of the salts in milk is present in or on the casein micelles, i.e., in colloidal particles. This undissolved salt is briefly called the colloidal, or

micellar, calcium phosphate, though it includes other components, i.e., K, Na, Mg, and citrate. The total amount is about 7 g/100 g dry casein. Part of it is to be considered as counterions. This is because the casein is negatively charged at the milk pH and is thus associated with positive counterions. This presumably involves the K, the Na, most or all of the Mg, and part of the Ca in the micelles. The rest, which is mainly calcium and phosphate together with a little magnesium and citrate, is present in a different state. Milk is supersaturated with respect to calcium phosphate and, accordingly, a large part of it is undissolved. Designating this part a precipitate would be incorrect. The calcium phosphate in the casein micelles consists of small noncrystalline regions and is, moreover, bound to the protein.

The molar ratio Ca/inorganic phosphorus in the micelles is high. Even if the part of the Ca present as counterions is subtracted, a ratio of about 1.5 remains for the calcium phosphate, i.e., as in tricalcium phosphate. That seems astonishing because we would expect a diphosphate (Ca/P $= 1$) at most, due to the pK_2 of phosphoric acid being about 7. Therefore, the phosphate esterified to serine residues of casein, i.e., the organic phosphate, is believed to participate in the colloidal phosphate as a result of which a ratio of ~ 1 would be found. However, the colloidal phosphate should not be seen as one of many known types of calcium phosphate. Moreover, it has a variable composition that depends on the ion atmosphere. For instance, as stated above, it contains Mg and citrate as well as traces of several ions, primarily Zn. In other words, the colloidal phosphate can be considered to have ion exchange properties.

2.2.4   Changes in Salts

The salts of milk are in dynamic equilibrium: among themselves in solution, between solution and colloidal phosphate, between solution and proteins. Changing external conditions of milk may cause alterations in equilibria. To be sure, there is no true equilibrium, especially not of calcium phosphates, but local or pseudoequilibria exist. The stress here is on the word dynamic.

Milk is saturated with respect to $CaHPO_4$ (solubility in milk is about 1.8 mmol/L, that of $Ca_3(PO_4)_2$ about 0.06). Furthermore, a small part of the citrate in milk is undissolved, as can be seen in Table 2.3. Milk is not saturated with respect to other salts (e.g., solubility of $MgHPO_4$ in water is approximately 12 mmol/L). Milk has a buffering capacity for some ions, primarily Ca^{2+}. This is mainly caused by the presence of undissolved colloidal phosphate, which can vary not only in quantity but also in composition, as a result of ion exchange. Imposing changes in ionic composition by temperature, pH, etc., has therefore a different effect on the salt solution of milk, where exchange with the micellar salts may occur, and that of whey or ultrafiltrate.

Table 2.8 gives examples of what will happen when some substances are

TABLE 2.8 Effect of Various Additions to Milk on Increase (+) or Decrease (−) of Ca and Inorganic Phosphate in Various States[1]

	Effect on the concentration of		
Substance added	Ca^{2+}	Dissolved Ca	Phosphate in the micelles
HCl	+0.2	+0.3	−0.2
NaOH	−0.1	−	+0.2
NaCl	+	+0.005	−
$CaCl_2$[2]	+0.3	+0.3	+0.4
Citric acid[2]	−0.1	+0.4	−0.2
Phosphoric acid[2]	−0.05	−0.1	+0.1
EDTA[2,3]	−	+	−

[1] As far as is known, the approximate magnitude of the change is given in moles Ca or phosphate per mole substance added, for a small addition.
[2] Plus as much NaOH as is needed to keep the pH constant.
[3] EDTA, ethylenediaminetetraacetic acid, a chelating agent.

added to milk. During storage of milk, some changes may occur. The milk loses CO_2; the original content in the udder is roughly twice that mentioned in Table 2.6. Enzyme action on dissolved phosphoric esters causes a decrease in pH and in $[Ca^{2+}]$, and an increase in the amount of colloidal phosphate. Lipolysis yields free fatty acids that decrease the pH and bind some Ca^{2+} ions. The calcium salts of fatty acids are poorly soluble (solubility product of fatty acid with $Ca^{2+} \approx 10^{-10}$ to 10^{-12} $mol^3 \cdot kg^{-3}$ and pK of fatty acids ≈ 4.7).

2.2.4.1 Acidity

The pH may change as a result of additions, by concentrating or heating the milk, and so forth. Microbial fermentation of lactose to lactic acid (Sections 2.1 and 11.1) is of great importance. The ensuing drop in pH causes a partial dissolution of the colloidal phosphate (Fig. 2.7), and a decrease of the negative charge of the proteins, which goes along with a decrease in the association with counterions. Moreover, a decrease in pH reduces the dissociation of weak acids, increases the $[Ca^{2+}]$ (see also Table 2.8, addition of HCl, and Fig. 2.9), and increases the ionic strength, presumably by more than corresponding to the amount of lactic acid formed. The acid production causes a decrease of the freezing point by about 2 mK per mmol lactic acid produced, and an increase of the electrical conductivity by ~ 4 mA $\cdot$ V^{-1} $\cdot$ m^{-1} per mmol lactic acid. Several lactic acid bacteria also break down citrate, and this would enhance the increase in $[Ca^{2+}]$. On the other hand, lactate associates to some extent with Ca^{2+}, and the increase in $[Ca^{2+}]$ on lactic fermentation will thus be less than would follow from the drop in pH.
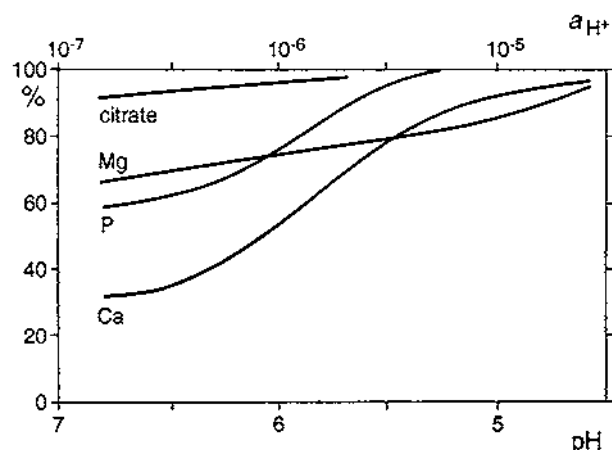
FIGURE 2.7   Approximate percentages of calcium, inorganic phosphate, magnesium, and citrate that are dissolved, as a function of the pH of milk.

2.2.4.2   Temperature Treatment

We should distinguish among three types of experiments.

  a. Measure the actual state in milk at various temperatures. For example, the dissociation constants are temperature-dependent. Very few direct measurements with respect to alterations in milk have been made, but we may try to infer the composition of the salt solution at different temperatures from the following experiment.
  b. Separate milk serum at various temperatures and investigate it at room temperature. It then follows that the pH of serum made at 0°C is ~0.1 unit higher, and that made at 93°C is ~0.5 unit lower, as compared to serum made at 20°C (Fig. 1.3).
  c. Heat milk at various temperatures for various times, cool to room temperature, and investigate. This is the most common type of experiment.

During heating, the most important change is that dissolved calcium and phosphate become partly insoluble and largely associate with the casein micelles. The additional colloidal phosphate formed has a molar ratio Ca/P ≈ 1. The reaction is roughly

$$Ca^{2+} + H_2PO_4^- \rightarrow CaHPO_4 + H^+$$
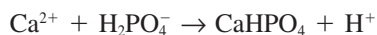
This implies that the milk becomes more acidic. (The drop in pH partly counterbalances the insolubilization of Ca and phosphate.) The reactions are slow and occur especially in a fairly narrow temperature range. Below 60°C, changes are

small, whereas above 80°C any further increase in temperature has little effect. The reactions reverse at room temperature, though very slowly (Fig. 2.8). At low temperature, the reverse occurs: after 24 h at 3°C, dissolved Ca is increased by about 7%, dissolved phosphate by about 4%, and Ca^{2+} concentration is also increased. The magnitude of all of these changes may vary.

2.2.4.3 Concentration

Concentration of milk by evaporation of water causes several changes, but it should be taken into account that heating is usually involved as well. The pH drops by about 0.3 unit for 2:1 concentration (i.e., concentrating to twice the original dry matter content) and by about 0.5 unit for 3:1 concentration. Again, the main cause of the changes is formation of additional calcium phosphate in the casein micelles. Accordingly, the Ca^{2+} concentration does not increase appreciably. The fractions of dissolved citrate, phosphate, and calcium decrease, but less than proportionally to concentration; for instance, dissolved calcium decreases from 40% to 30% for 2.5:1 concentration. This can be attributed partly to the pH decrease, partly to the increase of the ionic strength, but otherwise the changes are poorly understood.

2.2.4.4 Calcium Ion Activity

The Ca^{2+} activity ($a_{Ca^{2+}}$) in milk is an important variable, especially for the stability of casein micelles. It differs markedly from the content of dissolved
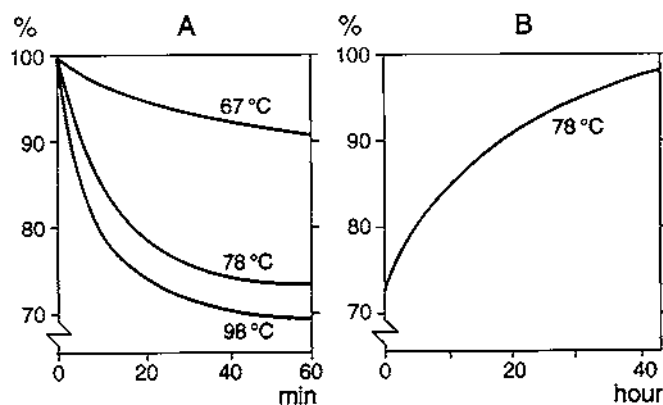
FIGURE 2.8 The amount of Ca in milk that is dissolved in percentage of the original amount. (A) Effect of the time of heating at various temperatures (determined after 1 h at 20°C). (B) Effect of holding at 20°C after heating for 30 min. Approximate examples. (From data by Jenness and Hilgeman, unpublished.)
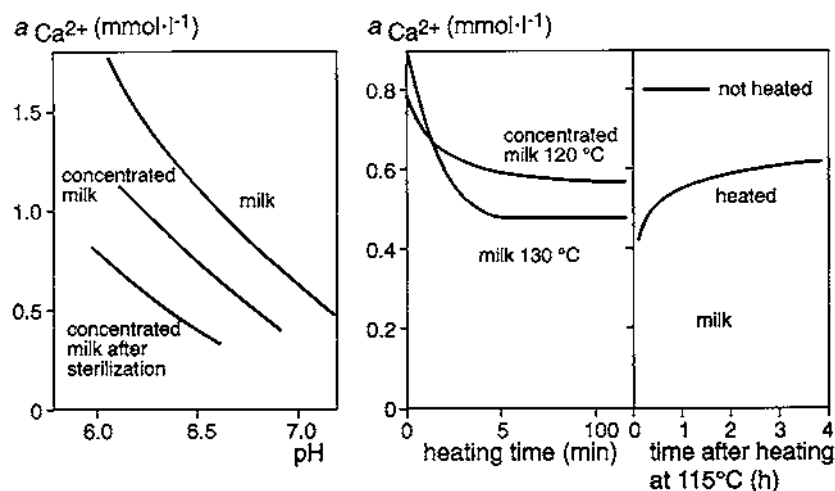
FIGURE 2.9 Effects of pH, heat treatment, concentration (by a factor of 2.6), and time after heating on calcium ion activity. All measurements at 20°C.

calcium. Because of this, direct determination of $a_{Ca^{2+}}$ is essential. This can be achieved by using a calcium ion–selective electrode, in much the same way as the pH is measured. Figure 2.9 summarizes the effect of some variables. Addition of sugar, e.g., sucrose, to milk (as is applied in the manufacture of sweetened condensed milk, ice cream mix, and other milk products) significantly increases $a_{Ca^{2+}}$ (expressed in mmol/kg water). For example, the activity coefficient of Ca^{2+} increases from 0.40 to 0.46 by the addition of 150 g sucrose to 1 L milk. Different milks vary widely in $a_{Ca^{2+}}$, from 0.6 to 1.6 mmol · L^{-1}. The variation usually correlates significantly with pH, i.e., the higher the a_{H^+} (the lower the pH), the higher the $a_{Ca^{2+}}$.

2.3   LIPIDS

Lipids are esters of fatty acids and related components that are soluble in nonpolar organic solvents and insoluble, or nearly so, in water. Alternatively, the term *fat* is used. But ''fat'' is usually considered to consist largely of a mixture of triglycerides, especially when the mixture is partly solid at room temperature.

Nearly all of the fat in milk is in fat globules. It can therefore be concentrated readily by means of gravity creaming, possibly followed by churning. Products rich in fat, such as cream and butter, have a specific and often desired flavor and texture. On the other hand, milk fat is prone to deterioration, leading to serious off-flavors. The consistency of high-fat products greatly depends on the

crystallization of the fat. In turn, crystallization behavior of milk fat depends on such factors as the widely varying fat composition.

In this section, composition and properties of lipids are discussed. Section 3.1 deals with specific aspects of milk fat globules.

2.3.1   Composition

Table 2.9 gives the main lipid classes of milk with their chemical structure and some of their properties. Note that about 98% of milk fat is a mixture of triacylglycerides. Various other lipids, some being present in trace amounts, are also dissolved in the fat. Most of the more polar lipids are in the fat globule membrane.

The chemical and physical properties of a lipid primarily depend on the kind of molecule. For example, triglycerides are different from lecithins or sterols. But each lipid class consists of many different kinds of molecules since it contains various fatty acid residues. Such a fatty acid pattern is an important factor in determining lipid properties, such as melting range, chemical reactivity, and nutritional value.

The following are the main variables among fatty acids in milk fat.

a. *Chain length*. Most fatty acids contain 4–18 carbon atoms; even-numbered acids are predominant.
b. *Number of double bonds*, in other words the degree of unsaturation. It mainly determines chemical reactivity, including proneness to autoxidation.
c. *Position of double bonds*, e.g., conjugated (—CH=CH—CH=CH—) or nonconjugated.
d. *Configuration of a double bond*. Each double bond can be either in the *cis* or *trans* position. The *cis* form is the common one in nature. Milk fat contains about 3 mol *% trans* acids, predominantly mono-unsaturated.
e. *Branching*. Nearly all of the fatty acids have an unbranched carbon chain. But some have a terminal —CH(CH$_3$)—CH$_3$ group.
f. Some fatty acids have a keto or hydroxy group.
g. On hydrolysis, the glycerides may yield some fatty alcohols and fatty aldehydes, in addition to fatty acids.

On heating, some of the fatty acids show chemical reactions. Residues of 3-ketoacids give rise to free methylketones, 4- and 5-hydroxy fatty acid residues give γ- and δ-lactones, respectively. These compounds are also present in fresh milk and are partly responsible for the characteristic flavor of milk fat. Higher quantities, which may arise from heat treatment or during long storage of dried milk, cause an atypical flavor. At still higher heating temperatures (e.g., 150°C) the position of part of the double bonds changes and some are transformed from

TABLE 2.9 Lipids of Fresh Milk[1]

Lipid class	Alcohol residue	Other constituent	$\overline{MW}$	Fatty acid residues			Percentage in milk fat (w/w)	Percentage of the lipid in		
				Number	$\bar{x}$	$\bar{y}$		Core of globule	Globule membrane	Milk plasma
Neutral glycerides:							98.7			
Triglycerides	Glycerol		728	3	14.4	0.35	98.3	~100	+	
Diglycerides	Glycerol		536	2	14.9	0.38	0.3	90?	10?	?
Monoglycerides	Glycerol		314	1	15.0	0.36	0.03	+	+	+
Free fatty acids	—		253		15.8	0.36	0.1	60	10?	30
Phospholipids[2]:		Phospho group					0.8	—	65	35
Lecithin	Glycerol	Choline	764	2	17.2	0.6	0.26			
Ph. ethanolamine[3]	Glycerol	Ethanolamine	742	2	17.9	1.0	0.28			
Ph. serine[3]	Glycerol	Serine	784	2	17.8	0.8	0.03			
Ph. inositide[4]	Glycerol	Inositol	855	2			0.04			
Plasmalogens	Glycerol	Choline[6]	~700	1[7]			0.02			
Sphingomyelin[5]	Sphingosine	Choline	770	1	19	0.2	0.16			
Cerebrosides[4,5]	Sphingosine	Hexose	770	1	20	0.2	0.1	—	70	30
Gangliosides[4,5]	Sphingosine	Hexose[8]	~1600	1			0.01	—	70?	30?
Sterols:							0.32	80	10	10
Cholesterol			387				0.30			
Cholesteryl esters	Cholesterol		642	1	16	0.4	0.02?	95?	5?	
Carotenoids + vitamin A	—						0.002			+

[1] Approximate average values; not complete; $\bar{x}$ = average number of carbon atoms; $\bar{y}$ = average number of double bonds.
[2] Approximately 1% is present as lysophosphatides.
[3] Phosphatidylethanolamine + Ph. serine = cephalin.
[4] Glycolipids.
[5] Sphingolipids.
[6] Or ethanolamine.
[7] Also a fatty aldehyde residue.
[8] Also neuraminic acid.

cis to *trans.* Interesterification or randomizing (i.e., interchange of fatty acid residues among their position in the triglyceride molecule) also occurs.

Table 2.10 gives data on the fatty acid pattern of some different lipid classes in milk; it is clear that there are large differences (see also Table 2.9).

Obviously, the fatty acid pattern of the triglycerides is highly variable and depends on several factors (Section 1.3). Nevertheless, milk fat can be characterized as follows:

a. Its fatty acid composition is very wide. Including fatty acids with keto or hydroxy groups, with an uneven number of C atoms, with branched carbon chain, the total number is some 250 different acid residues. Of these, 11 amount to over 1 mol % of the mixture of fatty acid residues.

b. It contains a relatively high proportion (15–20 mol %) of short-chain fatty acid residues, with 4–10 carbon atoms. This is typical of milk fat of ruminant species.

c. The proportion of saturated fatty acid residues is high, e.g., 70 mol % ($\approx$63% w/w).

d. Oleic acid is the most abundant of the unsaturated fatty acid residues (about 70%).

e. The other unsaturated fatty acid residues are present in a wide variety of chain length as well as number, position, and configuration of the double bonds.

Since the various lipids are unevenly distributed among the physical fractions of milk (Table 2.9), the fat composition of different milk products varies. The largest differences originate from variations in the amount of material from the fat globule membranes. Examples are given in Table 2.11. Anhydrous milk fat is prepared from butter by melting it, and by separating and drying the oil layer obtained; its composition is virtually equal to the fat in the core of the milk fat globules.

Milk fat can be modified chemically by interesterification (which occurs also somewhat during intense heating) and by hydrogenation (hardening), but the products obtained can no longer be called milk fat. Milk fat can be fractionated into high and low melting portions by letting it partly crystallize (Section 19.4). In milk fat, changes can occur by autoxidation (Section 2.3.3), lipolysis (Section 3.1.5), and intense heating (see above).

2.3.2 Some Properties

The properties of the individual lipid classes will be discussed briefly.

2.3.2.1 Triglycerides

Triglycerides make up the bulk (mostly more than 98%) of the lipids and, accordingly, largely determine the properties of milk fat. These properties vary with

TABLE 2.10 Fatty Acids in Milk Fat[1]

Acid	Notation[2]		Melting point (°C)	Solubility[3] (g/L)	Composition (in mol %) of			Percentage in 3 position
	x	y			Neutral glycerides[4]	Phospholipids	Free fatty acids[5]	
Saturated:					69 (57–80)	45	72	
Butyric	4	0	−8	Miscible	8.5 (7–14)	—	14.5	97
Caproic	6	0	−4	174	4.0 (2–7)	—	4.5	84
Caprylic	8	0	16	58	1.8 (1–3.5)	0.2	2	45
Capric	10	0	31	17	3.0 (1.5–5)	0.2	2	33
Lauric	12	0	44	5.6	3.6 (2.5–7)	0.5		26
Myristic	14	0	54	1.6	10.5 (8–15)	3	9	17
Palmitic	16	0	63	0.49	23.5 (20–32)	19	21	12
Stearic	18	0	70	0.14	10.0 (6–13)	12	13	22
Odd-numbered					2.5 (1.5–3.5)	4.5	2.5	7
Branched					1.1 (0.7–1.8)	0.7	1	
Other					0.7 (0.3–2)	5		
Monoene:					27 (18–36)	41	23	
Palmitoleic	16	1 Δ^9[6]			1.4	?	1?	23
Oleic	18	1 Δ^9[6]	16	0.42	21 (13–28)	38	20	32
Other					5.5	3	3.5	

Lipid class	x	y, position		%	%	%	%
Diene:				2.5 (1–4.3)	8		
Linoleic	18	2 Δ9,12[6]	— 5	1.8	8	2.3	20
Other				0.7	0.2		
Polyene:				0.8 (0.4–2)	4		
α-Linolenic	18	3 Δ9,12,15[6]	−12	0.4	2		29
Other				0.4	2		
Keto				0.3	?		
Hydroxy				0.3	?		
Fatty alcohol				0.01	0.15		
Fatty aldehyde				0.02	0.01		
Unclassified					2	1	

[1] Properties, approximate average fatty acid composition of some lipid classes, and average percentage of each fatty acid residue esterified in the 3 position of the triglycerides.

[2] x = number of C atoms; y = number of double bonds; Δ refers to the position in the carbon chain: $\Delta 9, 12$, for instance, indicates that the two double bonds occur at the ninth and twelfth bonds, counting from the carboxyl group.

[3] Critical micelle concentration in the presence of Na^+ ions.

[4] In parentheses is the approximate range.

[5] Free fatty acids liberated by the action of milk lipase.

[6] All *cis*.

TABLE 2.11 Approximate Content of Lipids in Some Milk Products

	Composition (% w/w)			
Product	Total fat	Phospholipids	Sterols	Free fatty acids
Separated milk	0.06	0.015	0.002	0.002
Milk	4	0.035	0.013	0.008
Cream	10	0.065	0.03	0.017
Cream	20	0.12	0.06	0.032
Cream	40	0.21	0.11	0.06
Buttermilk from 20% cream	0.4	0.07	0.005	0.002[1]
Buttermilk from 40% cream	0.6	0.13	0.011	0.002[1]
Butter (unsalted)	82	0.25	0.21	0.12[1]
Anhydrous milk fat	>99.8	0.00	0.25	0.15[1]

[1] Higher if the cream has been subject to lipolysis, especially after its separation.

the fatty acid composition. Since the number of different fatty acid residues is great, the number of different triglycerides is still much greater. The 11 major fatty acid residues alone would yield 11^3, or 1331, different triglycerides. Assuming that any other minor fatty acid residue would not appear more than once in a triglyceride molecule, we arrive at 10^5 different molecules at least. Moreover, there is presumably no single triglyceride species present in a concentration over 2 mol %. Clearly, milk fat shows a wide compositional range.

The distribution of fatty acid residues over the positions in the triglyceride molecule is far from random. For example, butyric and caproic acid are largely in the 3 and stearic acid in the 1 position (see also Table 2.10).

Position of the fatty acid residues in the triglyceride molecules determines what fatty acids are predominantly liberated by lipolytic enzymes (i.e., the acids in the 1 and 3 positions), and it considerably affects the crystallization behavior of milk fat. Most other properties depend only on fatty acid composition.

Triglycerides are very apolar and not surface-active. In the liquid state they act as a solvent for many other apolar substances, including sterols, carotenoids, and tocopherol. A small amount of water (about 0.15% at room temperature) dissolves in liquid milk fat. Some physical properties are given in Table 2.12.

2.3.2.2 Di- and Monoglycerides

Some of these occur in fresh milk fat. Lipolysis increases their quantities. Diglycerides are apolar and do not differ much from triglycerides in properties. Monoglycerides, present in far smaller quantities, are fairly polar; they are surface-active and thus accumulate at an oil–water interface.

TABLE 2.12 Physical Properties of Milk Fat

Temperature (°C)	Density (kg · m^{-3})	Refractive index at $\lambda = 0.589$ μm	Viscosity (mPa · s)	Solubility of water (% w/w)
10	922	(1.465)		(0.11)
20	915	1.462	70.8	(0.14)
30	909	1.458	45.7	0.17
40	902	1.454	30.9	0.20
50	895	1.451	22.1	0.24
60	889	(1.447)	16.6	0.27
70	882	(1.444)	12.5	0.32
80	876	(1.440)	9.8	0.36
90	(869)	(1.436)	7.6	0.41
100	(863)	(1.433)	6.2	0.46

Note: Some average results for liquid fat. Values between brackets are based on extrapolation.
Refractive index n_D (i.e., n at $\lambda = 0.589$ μm) is mostly 1.452–1.457 at 40°C.
Density varies by $< 1\%$ with composition.
Heat conductivity: ~0.17 J · m^{-1} · s^{-1} · K^{-1} at room temperature.
Specific heat: ~2100 J · kg^{-1} · K^{-1} at 40°C.
Total heat of fusion: ~85–100 J · g^{-1}.
Total melting dilatation: ~0.055–0.06 ml · g^{-1}.
Dielectric constant: ~3.1, but greatly depending on frequency.
Solubility of air in (liquid) fat: ~87 ml · kg^{-1}, i.e., ~28 ml O_2 and ~59 ml N_2, all at room temperature in equilibrium with air at atmospheric pressure. Quantity of O_2 in fat ~0.004% w/w (liquid fat in equilibrium with air). Solubility of air in solid fat is negligible.

2.3.2.3 Free Fatty Acids

These already occur in fresh milk and lipolysis increases their amount. The shorter acids are somewhat soluble in water. Fatty acids can, of course, dissociate into ions; their pK is about 4.8. In milk plasma, they are thus predominantly in the ionized form (i.e., as soaps), and these are much more soluble in pure water than the pure fatty acids are. Table 2.10 gives solubilities. Fatty acids dissolve well in oil, though only in the nonionized form; moreover, they tend to associate into dimers. Obviously, the partition of the acids over the oil and water fractions is rather intricate. All in all, it means that the shorter acids (C_4 and C_6) are predominantly in the plasma, the longer ones (from C_{14}) in the fat. The other acids are distributed between both fractions, though more go into the fat with decreasing pH (i.e., with ionization becoming weaker). This is all of much importance because it is the shorter acids that are responsible for the soapy-rancid flavor perceived after lipolysis. All this becomes even more complicated since the fatty acids, especially the long-chain ones, are surface-active and tend to accumulate in the oil–water interface.

2.3.2.4 Compound Lipids

Most abundant in milk are the phospholipids or phosphatides. Most of these have two charged groups (an acid and a basic one) and therefore are fairly polar. They do not dissolve well in water or oil, but form micelles in either liquid. They are highly surface-active and tend to associate with several proteins to yield lipoproteins. In milk, the compound lipids are largely in the fat globule membrane; in plasma, they are present in lipoprotein particles or ''milk microsomes.''

2.3.2.5 Unsaponifiable Lipids

Unsaponifiable lipids in milk consist largely of cholesterol, which is fairly apolar and associates easily with phospholipids; accordingly, part of cholesterol is in the fat globule membrane. A fraction of the cholesterol is esterified to a fatty acid (i.e., actually being saponifiable). Carotenoids are responsible for the yellow color of milk fat.

2.3.3 Autoxidation

The double bonds in a fatty acid or a fatty acid residue can oxidize. From the oxidation products obtained, several components can be formed. Some of these can be perceived in exceptionally low concentrations and thereby cause off-flavors, including tallowy, fatty, fishy, metallic, and cardboard-like. Off-flavor development can cause problems in beverage milk, sour-cream buttermilk, cream, and especially long-keeping high-fat products like butter and whole-milk powder. The complex of reactions involved is highly intricate, and there are several complicating factors. Following is a simplified summary account of the reactions involved.

   Molecular oxygen (i.e., triplet oxygen, $^3O_2$, with two unpaired electrons in the $2p\pi$ orbital) exists in a relatively unreactive state. The following oxides are much more reactive:

   a.   Singlet oxygen $^1O_2$
   b.   Superoxide anion radical O_2^-
   c.   Hydroxyl radical OH·

Singlet oxygen is the principal agent initiating oxidation of fat. This is because it is highly electrophilic: one of the oxygen atoms has two paired $2p\pi$ electrons, but the other has none in the $2p\pi$ orbital. It can readily react with a double bond to yield a hydroperoxide while shifting the double bond (Fig. 2.10, reaction 1b).

   The question now is how singlet oxygen (or one of the other reactive species) can be generated. In milk, there are a few pathways for $^1O_2$ to be formed:

   a.   As a result of photooxidation, i.e., a reaction between triplet oxygen and riboflavin, excited by light

b. By action of peroxidase and/or xanthine oxidase
c. By means of Cu^{2+} and ascorbic acid
d. By a reaction between the superoxide anion radical and H_2O_2
e. It can be formed by degradation of hydroperoxides during autoxidation of the fat

A simplified outline of fat oxidation is given in Figure 2.10. First of all, peroxide radicals should be formed (''initiation''). There are several pathways to achieve this. Usually the initiation is very slow until hydroperoxides are formed. The production of singlet oxygen presumably is crucial in the initiation. In milk, the main catalyst with respect to reaction 1c in Figure 2.10 probably is Cu^{2+}.

Then a chain reaction sets in (''propagation''). It keeps itself going; hence the name autoxidation. Now, the concentration of hydroperoxides increases significantly. If a suitable catalyst is available, some of the hydroperoxides can follow reaction 1c and thereby facilitate the initiation.

Occasionally, radicals react with each other to yield stable final products (in which fatty acid residues become covalently connected), thereby terminating the reaction (''termination'').

The formed hydroperoxides have no flavor. But they are fairly unstable and can break down in various ways to form unsaturated ketones and aldehydes, some (especially those with the group —CH=CH—CH=CH—COH) having a very strong flavor; i.e., they may have a threshold concentration as low as 10^{-3} ppm (see also Section 2.6).

Many other reactions occur during fat oxidation in milk. Moreover, the ratio of the reaction rates depends significantly on such conditions as temperature. Consequently, the reaction products can also vary in character. It implies that the off-flavors developed under certain conditions (e.g., high temperature) do not always correlate satisfactorily with those under other conditions, e.g., low temperature. The rule is that higher temperature leads to more rapid development of defects. The rate at which off-flavors develop especially depends on the extent of unsaturation. For example, the reaction rates for C_{18} acids with one, two, and three double bonds roughly are in the proportion $1:30:80$.

Antioxidants can block the chain reaction or prevent the initiation. However, they are consumed in this process. Natural antioxidants are tocopherol (which reacts with the radicals) and β-carotene, which can react with singlet oxygen. Several synthetic antioxidants have been made as well. So-called synergists (e.g., phospholipids dissolved in fat) enhance the action of antioxidants. Other substances, such as citrate, can react with the catalyzing metal ions.

Under practical conditions, autoxidation usually takes some time to set in. Initially, the antioxidants are consumed; after this has been achieved, peroxides

1. *Initiation*, e.g.

   a. Formation of singlet oxygen $^1O_2$

   $$|\overset{\cdot}{\underline{O}}-\overset{\cdot}{\underline{O}}| \longrightarrow \oplus \overline{\underline{O}}-\overline{\underline{O}}| \ominus$$
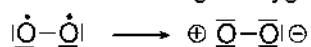
   b. Formation of peroxide

   c. Formation of radical

   $$ROOH \xrightarrow{\text{catalyst}} ROO\cdot + H\cdot$$

2. *Chain reaction*

   $ROO\cdot + RH \longrightarrow ROOH + R\cdot$ (slow)

   $R\cdot + {}^3O_2 \longrightarrow ROO\cdot$ (fast)

   ―――――――――――――――――――――――   +

   $RH + {}^3O_2 \longrightarrow ROOH$

3. *Termination*, e.g.

   $ROO\cdot + R\cdot \longrightarrow ROOR$

   $R\cdot + R\cdot \longrightarrow R-R$
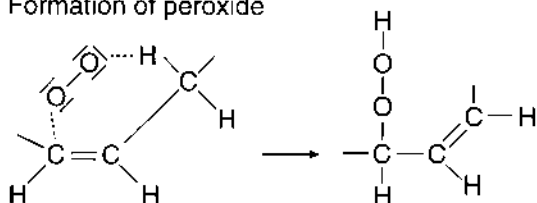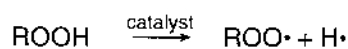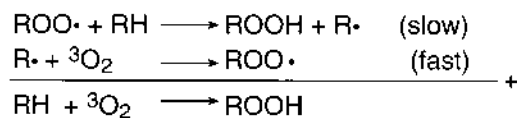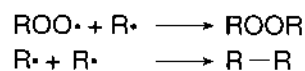
4. *Breakdown of hydroperoxides*

   $ROOH \longrightarrow$ unsaturated aldehydes and ketones $(C_6 - C_{11})$,

   e.g.
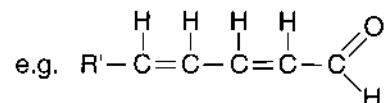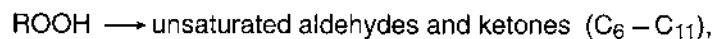
FIGURE 2.10   Approximate profile of the autoxidation reaction of unsaturated fatty acid residues.

are first liberated and subsequently are broken down to form perceptible amounts of flavor products, as has been stated above (see Fig. 2.11).

Autoxidation of milk fat in milk and milk products usually starts with the phospholipids of the fat globule membrane. These lipids have highly unsaturated fatty acid residues (Tables 2.9 and 2.10). Moreover, Cu, the main catalyst, can
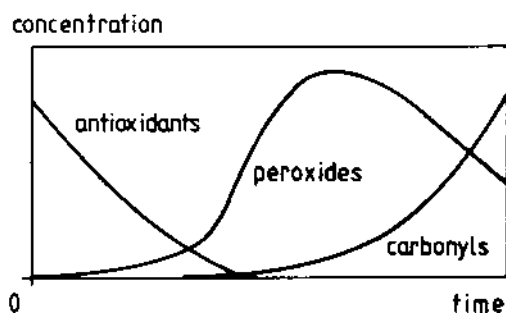
FIGURE 2.11 Relative concentrations of antioxidants, hydroperoxides, and free carbonyl components during autoxidation of a fat. Highly schematic, not to scale.

be in the membrane of the fat globules but not in the core, which contains the triglycerides. Copper is particularly active as a catalyst if phospholipids are present; moreover, at least in milk and buttermilk, a little ascorbic acid is needed. Cu entering the milk by contamination during or after milking is much more active as a catalyst than natural Cu. There are large variations in susceptibility to autoxidation of fat among different lots of milk. In some milks, contamination with as little as 5 µg Cu per kg suffices for an oxidized flavor to develop, whereas in others 200 µg·kg^{-1} is insufficient. Some workers assume that oxidation is spontaneous in some milks. Minute Cu contamination (e.g., 10 µg · kg^{-1}) is, however, hardly avoidable in practice. Incidentally, Fe can also be active as a catalyst, but not in the presence of proteins, i.e., not in milk.

Autoxidation of fat in milk can also be enhanced by exposure to light of short wavelengths. Putting a bottle of milk for 10 min in sunlight usually suffices to subsequently produce a distinct tallowy flavor. Other light-induced off-flavors can also develop, i.e. flavors in which lipid oxidation is not involved (Section 2.6). Riboflavin is an essential factor in light-induced autoxidation.

Early lactation milk on the average is more prone to develop flavors caused by fat oxidation. The natural variation in proneness may be ascribed to variations in redox potential, concentrations of tocopherol (antioxidant), ascorbic acid and oxidases (peroxidase, xanthine oxidase). Variation in activity of superoxide dismutase may also be of importance. This enzyme catalyzes the decomposition of superoxide anion (Section 2.5), and thereby acts as an antioxidant.

Several treatments can affect autoxidation reactions:

a. The role of Cu in autoxidation primarily depends on its concentration in the fat globule membrane. The concentration of natural Cu in the membrane presumably is too low (the amount being constant at about

10 μg/100 g fat, even if Cu concentration in the milk is high) to cause significant oxidation. Cooling of the milk (e.g., keeping it at 5°C for 3 h) causes a further decrease, since nearly half of the natural Cu moves to the plasma.

b.   In milk, 1% to 10% of ''added'' Cu goes to the membrane, the percentage increasing with the quantity added.

c.   Heating of the milk causes part of the copper to move from the plasma to the membrane. Heating for 15 s at 72°C has a significant effect; 15 s at 90°C has much more. The Cu content of the membrane may increase by 15-fold. Heating only the cream results in a smaller increase because then a smaller amount of Cu per unit mass of fat globules is available. To be sure, all of this copper does not necessarily have full catalytic activity (see item f).

d.   Souring milk or cream causes 30% to 40% of ''added'' Cu to move from the plasma to the fat globule membrane. This may explain why ripened-cream butter is more prone to autoxidation. But the oxidation reaction itself may also be faster at lower pH.

e.   Heating of the cream (e.g., 15 s at 83°C) before acidification largely prevents the transport of Cu, mentioned in item d. The explanation is uncertain, but the next point may be the cause.

f.   Intensive pasteurization causes exposure of more sulfhydryl groups, and especially formation of free H_2S. H_2S removes Cu^{2+} ions because CuS has a solubility product as small as 10^{-47} mol$^2 \cdot$ L^{-2}. Presumably, Maillard products may also act as antioxidants. All in all, intensive heating markedly inhibits autoxidation.

g.   Heating can cause inactivation of superoxide dismutase (EC 1.15.1.1), which probably is an important antioxidant. Moderate heating of milk increases the proneness to autoxidation, maybe also because of Cu migration (item c).

h.   Homogenized milk is much less prone to autoxidation, even if induced by light. The change in surface layer of the fat globules must be the cause, but the explanation is unknown.

i.   In general, the rate of autoxidation increases with increase in temperature ($Q_{10} \approx 2$). This also holds for many milk products. In fresh raw milk, however, oxidized flavor develops more quickly if temperature is lower. The explanation is not certain, but the activity of superoxide dismutase probably is involved.

j.   The rate of autoxidation reactions in dried products depends on water activity (a_w); see Figure 9.4. Clearly, water is an antioxidant. This is a factor in milk powder where tallowy flavors develop faster if the water content, hence a_w, is lower. At low a_w, ascorbic acid is not needed to cause autoxidation, and tocopherols do not act as inhibitors.

k. The oxygen content becomes a limiting factor if it is below about 0.8 ml O_2/100 ml fat, corresponding to an oxygen pressure of 0.1 bar. Such low levels of O_2 can only be achieved in fermented milks and cheese, or in products in hermetically sealed packages, e.g., milk powder packed in tins.

2.3.4 Crystallization

Crystallization of fats (triglycerides) is a complicated phenomenon, especially for milk fat with its very broad composition.

2.3.4.1 Melting Range

Fatty acids have widely varying melting points. This is reflected in the melting range of the fats in which the acids have been esterified. The shorter the chain length and the greater the number of double bonds, the lower the melting point (Table 2.10). But other variables also affect the melting point:

a. Odd number of carbon atoms: odd-numbered ones melt $\sim$5 K lower than fits the series with an even number of C atoms.
b. Branching: may cause a decrease by 1–40 K as compared to un-branched carbon chains.
c. Position of double bond: may make a difference up to about 20 K.
d. *Trans*: 20–30 K higher than *cis.*
e. Conjugated: $\sim$25 K higher than nonconjugated.

The melting point of a triglyceride molecule also depends on the distribution of the fatty acid residues over the three positions. A strongly asymmetric triglyceride (e.g., PPB where P = palmitic acid and B = butyric acid) has a lower melting point than a symmetric one with the same fatty acid residues (e.g., PBP).

Milk fat is a mixture of many widely different triglycerides (Section 2.3.2) with different melting points. The multicomponent fat thus has a wide melting range, as is shown in Figure 2.12. Between $-30°C$ and $+40°C$, milk fat usually consists of liquid as well as solid fat, i.e., oil with various crystals. Every individual triglyceride is fully liquid far below its melting point. For example, the highest melting triglyceride is tristearate; its melting point is 72°C, i.e., about 35 K above the final melting point of milk fat. The ''solid'' triglycerides are thus dissolved in the liquid fat. The solubility of a single triglyceride in oil is consistent with the thermodynamic theory for perfect solutions. But if several triglycerides crystallize, they may interfere with each other's solubility. In turn, this effect on the solubility depends on external conditions, as will become clear below. That explains why the melting curve of milk fat cannot be simply derived from its composition. Naturally, a higher content of high-melting triglycerides causes a higher content of solid fat at, say, room temperature.

% solid fat
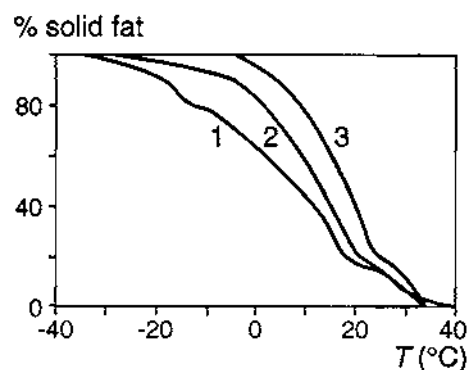
FIGURE 2.12 Melting curves of milk fat. 1. ''Summer'' fat, slowly cooled. 2. Same fat, rapidly cooled. 3. ''Winter'' fat, rapidly cooled. After J. Hannewijk and A.J. Haighton, *Neth. Milk Dairy J.* **11** (1957) 304.

Solidification curves depend even more closely on external conditions than melting curves do. This is because supercooling may occur, as becomes clear below.

2.3.4.2 Nucleation

A substance cannot crystallize unless nuclei have formed, i.e., tiny embryonic crystals, just large enough to escape immediately dissolving again. (The solubility of a small particle increases as its radius of curvature decreases.) Homogeneous nucleation (i.e., the formation of nuclei in a pure liquid) often requires considerable supercooling to form nuclei within, say, a few hours. In fats, a supercooling of about 35 K below the final melting point is needed. But nucleation is usually heterogeneous, i.e., it takes place at the surfaces of very small ''contaminating particles.'' Such particles are called catalytic impurities. As a rule, the number of impurities that catalyze nucleation (N) significantly increases with decreasing temperature.

In milk fat, if present in bulk (i.e., as a continuous mass), a supercooling of a few, say, 5 K, causes sufficient catalytic impurities to induce crystallization. As soon as fat crystals have been formed, they can, in turn, act as catalytic impurities for other triglycerides. For this to happen, a very small supercooling suffices. This is because of epitaxy, i.e., the crystal lattice of the molecules to crystallize almost fits on that of crystals already present. Milk fat in bulk may indeed show little hysteresis between solidification and melting curves (Fig. 2.13A).

The situation may be very different if the fat has been emulsified. In bulk fat, some 10^3 catalytic impurities per gram would suffice to ensure rapid crystallization. But in milk, 1 g of fat is divided over about 10^{11} globules, in homogenized
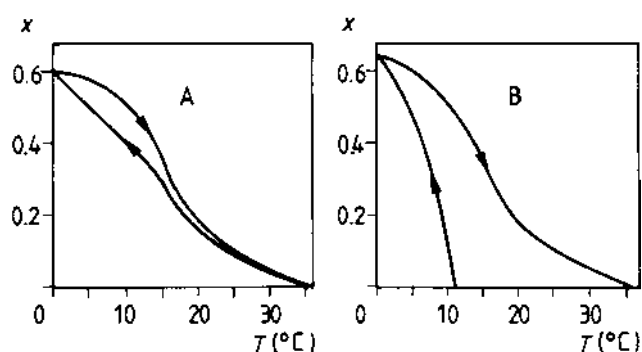
FIGURE 2.13 Examples of the proportion of fat being solid (*x*) after 24 h cooling to temperature *T*, and after warming again (after keeping it at 0°C). (A) Fat in bulk. (B) Same fat in recombined cream. Examples after P. Walstra and E.C.H. van Beresteyn, *Neth. Milk Dairy J.* **29** (1975) 35.

milk even over $>10^{14}$ globules. In each of those at least one nucleus must be formed. Consequently, considerable supercooling may be necessary, and significant hysteresis between solidification and melting curves occurs (Fig. 2.13B). Naturally, the magnitude of this effect depends on globule size, i.e., the supercooling should be deeper if the globules are smaller. If there are *B* catalytic impurities per ml of fat and if globule volume is *v* ml, then

$$y_{max} = 1 - e^{-vB}$$

where *y* is the proportion of the fat in globules containing one or more crystals. Variable *B* greatly depends on temperature. For milk fat, *B* roughly doubles for each 1.75 K lowering of temperature. In a certain temperature range, part of the fat may thus be devoid of any crystal present if the fat has been finely emulsified. In principle, each globule should eventually contain crystals. In practice, however, no further changes occur after, say, 24 h. Crystallization in finely divided fat is slower than in fat present in bulk. Figure 2.14A and B gives examples of supercooling for milk fat of a certain composition.

Presumably, micelles or crystals of monoglycerides are the main catalytic impurities in milk fat. For instance, less deep supercooling is needed with increasing lipolysis, implying higher values of *B*.

Another important phenomenon is secundary nucleation, which appears to be prominent in triglyceride mixtures, especially in milk fat. It means that as soon as a crystal is formed from a nucleus, other crystals form rapidly in the vicinity of the first one. This happens if the supercooling is fairly deep. It implies a high concentration of nuclei, i.e., a high number of crystals per unit volume.
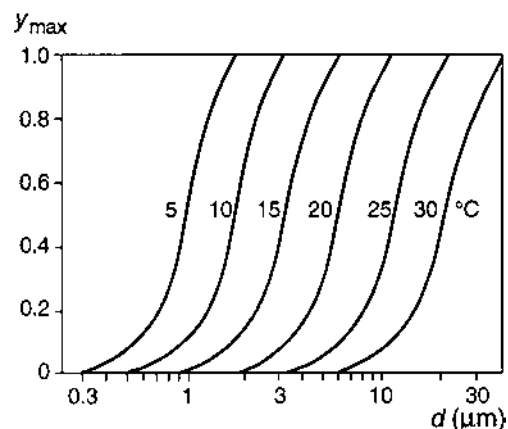
FIGURE 2.14(A) Examples of the fraction of milk fat present in fat globules that eventually contain solid fat (y_{max}) as a function of the globule diameter (d) and temperature. Example after P. Walstra and E.C.H. Beresteyn, *Neth. Milk Dairy J.* **29** (1975) 35.

2.3.4.3 Crystal Growth

In milk fat, growth of nuclei or crystals is very slow. The large, elongated, and flexible triglyceride molecules take a long time before obtaining the correct position and conformation to fit into the crystal lattice. Before a molecule actually fits in, it often diffuses away again. Moreover, there are many competing molecules, which are so similar to those in the crystal that they almost fit into its lattice. They have to diffuse out again before a properly fitting molecule can occupy a site in the crystal lattice.

For isothermal crystallization of milk fat at 25°C, it may take 1–2 h before half of the final amount is crystallized. This $t_{0.5}$ is roughly halved for each 5–6 K temperature decrease. This is, of course, because a lower temperature implies a greater supersaturation. However, crystallization rates in practice depend on conditions such as crystal size and the rate of removal of the heat of crystallization. Moreover, the nucleation rate may be involved.

2.3.4.4 Polymorphism

Triglycerides, like most molecules with long aliphatic chains, can crystallize in three different modifications, denoted as α, β′, and β. Each modification is characterized by its crystal lattice type (i.e., the mode of packing of the molecules), including the corresponding distances between layers of molecules; therefore, the modifications can be identified by X-ray diffraction. In the order α, β′, and β, the melting points increase (for tristearate, e.g., 55, 64, and 72°C), as do the
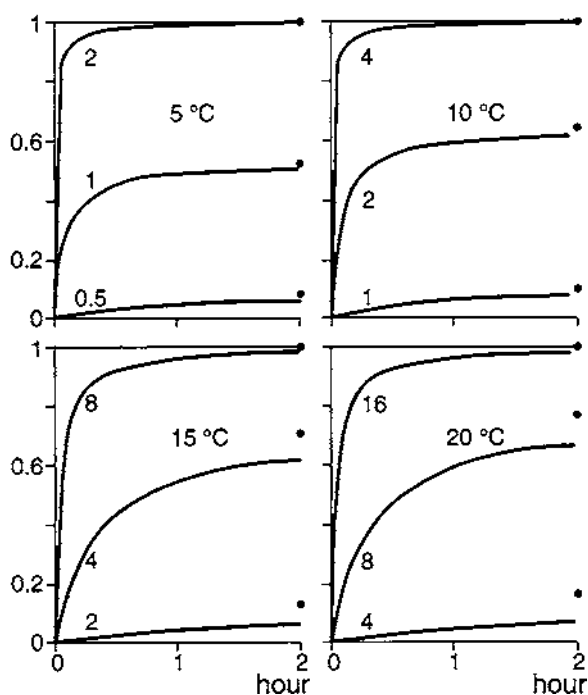
FIGURE 2.14(B) Examples of the fraction of milk fat present in globules that contain solid fat (ordinate) as a function of the time after cooling (abscissa) to 5, 10, 15, and 20°C, in globules of various diameter (in μm, indicated near the curves). The dot refers to the value after 24 h (y_{max}). Example after P. Walstra and E.C.H. van Beresteyn, *Neth. Milk Dairy J.* **29** (1975) 35.

enthalpy of fusion and the density of the crystals. This implies that closeness and intricacy of fit of the molecules in the lattice increase and their freedom of motion decreases in the same order. The α and β′ modifications are metastable. Transitions can only take place according to the scheme
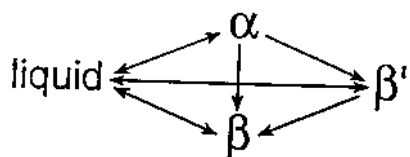
Other transitions cannot occur.

Nucleation usually occurs in the α modification. Mostly, after a little while, transition to a stabler modification occurs. In milk fat that has just started to crystallize, we can observe that warming at first causes melting, followed by a

second solidification (in a stabler modification), and eventually melting occurs. In most fats the α modification has a very short lifetime, whereas β′ may persist for a longer time. But in milk fat, α crystals can be very persistent (this is because of formation of compound crystals; see below), and especially at lower temperatures both α and β′ modifications are found. The final melting points in milk fat are approximately 22°C, 30°C, and 36°C for α, β′, and β, respectively. In other words, in milk fat no crystals in the α modification can exist above ~20°C and no β′ crystals above ~30°C.

The importance of the polymorphism is that a partially crystallized fat essentially is never in equilibrium. Rearrangement of crystal composition would always be expected to occur, especially at somewhat higher temperatures and during temperature fluctuations.

2.3.4.5   Compound Crystals

Two or more different components may occur together in one crystal. In some of these compound or mixed crystals, the components may occur in all proportions (called solid solutions); in others, the compositional range is restricted. In fats, compound crystals readily occur.

Milk fat exhibits extensive mixed crystallization, probably because of the very great number of distinct, though similar, triglyceride molecules. Thereby the total supersaturation with respect to the molecules that can form a compound crystal is much higher than that with respect to each of the individual triglyceride molecules separately. Compound crystals are formed easily and abundantly in the α, and usually not in the β, modification. In β crystals, the molecular packing is so dense that different kinds of molecule cannot fit in the same crystal lattice. Cooling the fat more rapidly to lower temperatures causes more compound crystals to be formed. These essentially are impure, i.e., less ordered, crystals. Accordingly, they have a lower heat of fusion than corresponding pure crystals. Although compound crystals form in principle in thermodynamic equilibrium, even a slight temperature difference causes a difference in equilibrium composition. Moreover, compound triglyceride crystals especially form in the unstable polymorphic modifications. All of this means that in practice true equilibrium never is reached (except possibly at temperatures above the final β′ melting point), and that polymorphic transitions and changes in compound crystals occur simultaneously.

The theory of compound crystals is fairly intricate and will not be discussed here. We give some important consequences of compound crystallization that are also noticed in actual practice:

a.   Compound crystals narrow the melting range.
b.   The temperature range at which most of the fat melts depends on the temperature at which solidification took place. It is found to be slightly above the latter temperature. Consecutive solidification at two tempera-

tures thus yields a differential melting curve [$-$d(solid fat)/dT against T] with two melting maxima.

c. Cooling in steps and slow cooling give less solid fat than rapid and direct cooling to the final temperature. Compare curves 1 and 2 in Figure 2.12.

d. Cooling to a lower temperature before bringing to the final temperature gives more solid fat than direct cooling to the latter. This is shown in the hysteresis at low temperatures in Figure 2.13A.

e. During keeping, slow rearrangement of crystal composition occurs since equilibrium has not been reached. Below 5°C, this is a very slow process; at higher temperatures, it can still take days.

f. Also after a change of temperature, crystallization takes a long time before reaching equilibrium. After lowering the temperature, the amount of solid fat may increase during several days. After increasing the temperature to below the final melting point, it may take up to a half-hour before melting has stopped.

All in all, the temperature history of the fat appears to have a significant effect on compound crystallization.

2.3.4.6 Size and Shape of Crystals

The geometrical form (habit) of a crystal should be distinguished from the above-mentioned modification. Each modification may include widely varying habits.

In most cases, rapid cooling of milk fat leads to formation of platelets. The ratio of the platelet sizes approximates $4:2:1$; platelet length $\approx 0.1-3$ μm; platelet concentration may be on the order of 10^{12} per gram of fat. In principle, the slower the cooling, the fewer crystals are formed and the larger they become. However, the secondary nucleation mentioned above always causes the formation of many, and thereby small, crystals, unless there is little supercooling. The slow changes mentioned above (polymorphic transition, change in compound crystals) and temperature fluctuations may cause larger crystals to grow at the expense of smaller ones. Large spherulites (spheres built of radial needles) up to 1 mm in diameter can be formed at small supercooling.

2.3.4.7 Rheological Aspects

Fat crystals suspended in oil attract each other because of van der Waals forces and only repel each other because of hard core repulsion (i.e., when they touch). Accordingly, they always flocculate and this causes formation of a continuous crystal network, in which the oil is held if sufficient crystals are present (a few percent by weight). The network causes the fat to have a certain firmness, which increases with increasing fraction of solid fat and depends also on size and shape of the crystals. Furthermore, rearrangement of crystal composition may occur

because often the crystalline phase is not in equilibrium. Crystals thus will grow locally and, in this way, flocculated crystals may fuse, i.e., become sintered. All of these changes are illustrated in Figure 2.15. Temperature fluctuations (at first melt part of the fat by warming, then crystallize it slowly by cooling) can also cause considerable sintering. Thereby the fat becomes much firmer because the bonds between the original elements of the network strengthen considerably. Strong deformation (e.g., a stress of 10 MPa) can cause the fat to flow and the sintered bonds to be broken locally. As a result, the firmness of the fat decreases. After keeping the fat for some time, strong bonds are formed again, so that part of the lost firmness is regained. In other words, the fat is more or less thixotropic.

The rheological aspects are of paramount importance to firmness and spreadability of butter. Most of the fat in butter is in a continuous mass and can form a continuous network. In cream, the fat is retained in single globules. As long as nothing happens, a crystal network can form in each globule of the cream, but not throughout the cream volume. Because of this, milk fat and butter have a certain stiffness, whereas high-fat cream is liquid.
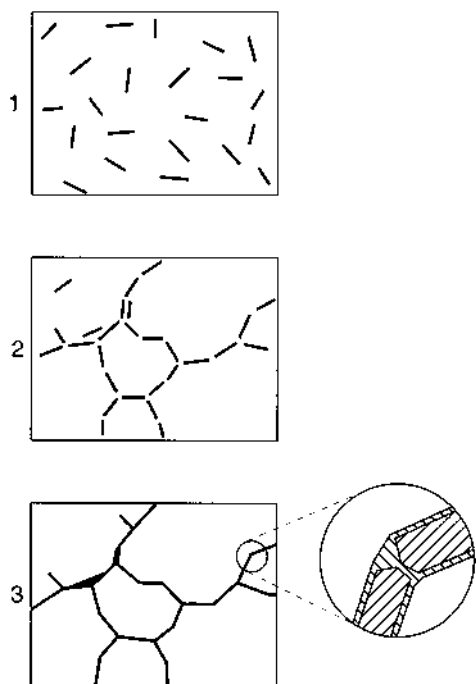
FIGURE 2.15   Various stages during crystallization showing flocculation and sintering of fat crystals. Highly schematic.

TABLE 2.13 Summary of Factors Affecting Crystallization of Milk Fat

		Effect on	
Factor	Melting curve[1]	Amount of solid fat	Crystal habit
Lower crystalli- zation tem- perature	Maximum at lower tem- perature	Greater	Smaller[3]
Faster cooling	Maximum at higher tem- perature	Generally greater	Smaller[3]
Cooling in steps	More than one maximum	Generally less	Often larger: spherulites[3]
Precooling to low tem- perature	Maximum at lower tem- perature	Greater	Somewhat smaller[3]
Keeping at not too low a tem- perature	Flatter	Generally greater	Larger; solid structures
Fat in globules rather than in bulk	Higher final melting tem- perature[2]	Low tempera- ture: greater / High tempera- ture: less	Smaller
Smaller globules		Generally less	Smaller

[1] Described as differential curve: $-d(\text{solid fat})/dT$ as a function of T, i.e., the amount of fat that melts within a small temperature interval.
[2] Because of variation in composition among fat globules.
[3] This does not apply to fat in globules.

2.3.4.8 Summary

Table 2.13 summarizes the influence that factors have on crystallization. Of course, the composition of the fat also has a considerable effect.

2.4 PROTEINS

2.4.1 Chemistry of Proteins

Proteins are made up of amino acids—more precisely, L-α-aminocarboxylic acids:

$$
\begin{array}{c}
NH_2 \\
| \\
R\text{----}C\text{----}CO_2H \\
| \\
H
\end{array}
$$
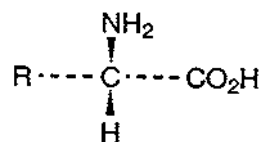
At neutral pH, the acids ionize to form $R \cdot CH(NH_3^+) \cdot CO_2^-$. They thus are so-called zwitterions, being at the same time acid and base. An amino group and a carboxyl group can react with each other to yield a *peptide linkage* while releasing a water molecule. The structure of the peptide linkage is intermediate between
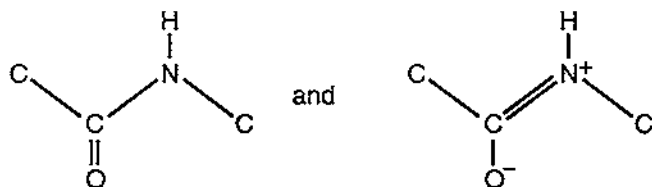
Because of this, the peptide bond has a dipole moment, and is planar and rigid, i.e., it remains in the *trans* configuration. Rotation is possible to some extent about the other bonds in the peptide chain (N—CR and CR—CO; Fig. 2.16), except for the N—CR bond in proline residues.

A linear peptide chain can be formed from a number of amino acids, as is shown in Figure 2.16. If such a chain is short, it is called peptide; if it is long, the term polypeptide or protein is applied. Most proteins contain at least 100 amino acid residues. There are 20 different natural amino acids, hence 20 different kinds of side chain R. Moreover, modifications of the side chain groups can occur (see below). Properties of amino acids are listed in Table 2.14.

2.4.1.1 Primary Structure

Primary structure is defined as the sequence of the different amino acid residues in the peptide chain. It is specific for every individual protein and is genetically determined. For most proteins genetic variants occur, i.e., one or two amino acid residues on a certain location in the chain differ (Section 1.3.2). Some of such changes can hardly be perceived, but in other cases it causes significant differences in protein properties, e.g., in solubility or in heat stability.

The specific properties of a protein thus are determined by the side chains R of the amino acids in the polypeptide chain (Table 2.14). The aliphatic apolar
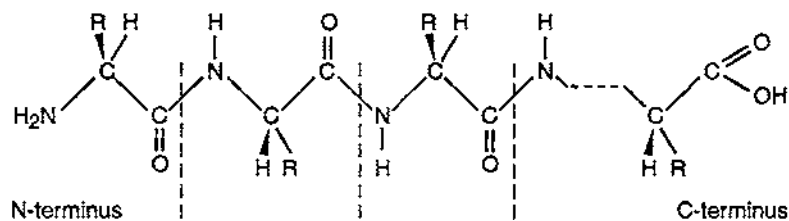
FIGURE 2.16 Peptide chain.

side chains are hardly reactive. Side chains with a carboxyl group (Asp, Glu) or an amino group (Lys, Arg) ionize at neutral pH to yield $-CO_2^-$ and $-NH_3^+$, respectively, and so do terminal carboxyls and amino groups; also His is partly protonated. The free carboxyls can be esterified. The free amino groups, especially those of lysine, can contribute to Maillard reactions (Section 6.2). Cysteine can react readily with another cysteine residue to yield cystine, and thereby form a covalent bond ($-S-S-$ linkage) between two peptide chains (intermolecular bond) or between two locations in one chain (intramolecular bond). This reaction occurs more readily at high pH, at which a greater number of the $-SH$ groups is dissociated into $-S^- + H^+$. To the free hydroxyls (Ser, Thr), acids (e.g., phosphoric acid) can be esterified. Proteins with phosphate ester groups are called phosphoproteins. In addition, saccharides can be linked to a hydroxyl group or an amide group, and this yields glycoproteins. Chromoproteins contain heavy metals, e.g., Fe or Mo; several enzymes belong to this group. All of these building blocks affect the properties of the proteins.

The associations of protein with lipids to yield so-called lipoproteins are of a different category. It mostly concerns noncovalent bonds, and the molar ratio of protein to lipid is variable. Most of the lipids involved can be removed by means of extraction with apolar liquids.

2.4.1.2 Conformation

Depending on the primary structure, several bonds including hydrogen bonds, hydrophobic interactions, and internal salt bridges can be formed between various parts of the long flexible peptide chain, which can thereby fold into a conformation specific to the protein involved. Locally, regular arrangements may be formed. Examples are the α helix and the β sheets that are built of a number of zigzag-like strands. All such arrangements are called *secondary structure*. The further steric arrangement of the peptide chain, including the parts with secondary structure, is called *tertiary structure*.
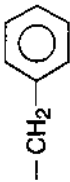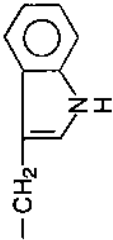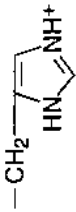
2.4.1.3 Some Properties

Some groups of a protein can be hydrated, especially charged groups and peptide bonds. Proteins thus bind some water, generally 10–20 g per 100 g of protein. This property should be distinguished from the ability of some proteins (e.g., gelatin) to swell in water, causing large quantities of water to be held or immobilized in the network of peptide chains.

A delicate combination of forces determines the final conformation of the polypeptide chain. The average hydrophobicity Φ (averaged over all amino acid residues present) is of major importance. (To be sure, there is no consensus of opinion on the values of Φ of the various amino acid residues.) If there are sufficient hydrophobic residues, folding of the chain into a roughly globular unit (called a globular protein) generally occurs in such a way that a hydrophobic

TABLE 2.14 Properties of Amino Acid Residues

Name of acid	Symbol	Side chain	Reactive group	pK'	Charge at pH 6.6	Φ^2
Glycine	Gly	—H				0
Alanine	Ala	—CH$_3$				3
Valine	Val	—CH(CH$_3$)CH$_3$				10
Leucine	Leu	—CH$_2$CH(CH$_3$)CH$_3$				12
Isoleucine	Ile	—CH(CH$_3$)CH$_2$CH$_3$				12
Serine	Ser	—CH$_2$OH	Hydroxyl			—
Threonine	Thr	—CHOHCH$_3$	Hydroxyl			—
Aspartic acid	Asp	—CH$_2$CO$_2^-$	Carboxyl	4.0	−1	—
Asparagine	Asn	—CH$_2$CONH$_2$	Amide			—
Glutamic acid	Glu	—CH$_2$CH$_2$CO$_2^-$	Carboxyl	4.5	−1	—
Glutamine	Gln	—CH$_2$CH$_2$CONH$_2$	Amide			—
Lysine	Lys	—(CH$_2$)$_4$NH$_3^+$	ε-Amino	10.5	+1	—
Arginine	Arg	—(CH$_2$)$_3$NHC(NH$_2$)$_2^+$	Guanidine	>12.5	+1	—
Cysteine	Cys	—CH$_2$SH	Thiol	8.5	0	?
Methionine	Met	—CH$_2$CH$_2$SCH$_3$	Thioether			?

Amino acid	Abbrev.	Side chain	Group	pK[1]	Charge	Hydrophobicity[2]
Phenylalanine	Phe	—CH$_2$(phenyl)	Phenyl			9
Tyrosine	Tyr	—CH$_2$(phenol)—OH	Phenol	9.6	0	?
Tryptophan	Trp	—CH$_2$(indole)	Indole			10
Histidine	His	—CH$_2$(imidazole) HN—NH$^+$	Imidazole	6.4	+$\frac{1}{2}$	—
Proline	Pro	(Note[3])				?
Phosphoserine	Serp	—CH$_2$O—PO$_3^{2-}$	Phosphoric acid	1.5, 6.5	−1$\frac{1}{2}$	—
Terminal groups	—	—CO$_2^-$	α-Carboxyl	3.6	−1	
—	—	—NH$_3^+$	α-Amino	7.6	+1	

[1] Approximate intrinsic ionization constant in unfolded peptide chains.

[2] Hydrophobicity in kJ per residue (−, value small or negative; ?, value uncertain).

[3] Secondary amino acid:

(proline structure: pyrrolidine ring with —CO$_2$H and NH)
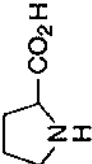
core is formed. In such a core hydrogen bonding can occur, which can lead to regular (i.e., secondary) structures, e.g., the α helix. Such structures often occupy only a limited proportion of the protein. Proline residues in particular prevent an α helix from being formed. Most of the outside of the protein molecule is fairly hydrophilic, and it holds most of the charged groups. All of these statements are not absolute because the primary structure may not allow formation of an ''ideal'' conformation of the protein, i.e., with all nonpolar side chains inside and all polar groups on the outside. In other words, shielded charges may be present in the inside and hydrophobic patches on the outside. If the average hydrophobicity is small and/or the protein molecule small, long stretches of chains (fibrous protein) rather than globular units tend to be formed.

Environmental conditions including temperature, pH, and ionic strength can affect the conformation of the protein. In some cases, a small change in conditions has a large effect. Resultant conformational changes can affect several properties of the protein, e.g., (a) the ability of certain groups of the protein to react with solutes, (b) the dissociation of ionizable groups, (c) the tendency toward association, and (d) the extent to which it becomes an accessible substrate to certain enzymes. It is important to note that an enzyme itself is a protein molecule and that its action greatly depends on its conformation.

2.4.1.4 Solubility

Most factors affecting the conformation of a protein also determine its solubility. If there is little water available compared to the amount of protein present or if the protein molecules are strongly crosslinked, the protein cannot dissolve. However, it may show swelling with water, and the amount of swelling is strongly correlated with the solubility. All proteins have electrically charged groups (Table 2.14), most of them having a greater number of negative than of positive groups at neutral pH. The charge causes the molecules to repel each other, which may contribute to the stability of the protein solution. The charge depends on the pH. At the so-called isoelectric pH, symbolized pI, the net charge is zero, and at this pH many proteins are virtually insoluble. At pH $>$ pI, the net charge is negative, and at pH $<$ pI it is positive. Protein solubility is strongly affected by ionic strength. Many proteins need at least a small amount of salt for dissolution (salting-in), but at high salt concentrations they are less soluble (salting-out). The presence of hydrophobic patches on the outside can cause hydrophobic interactions between the protein molecules to occur. Because of this, globular proteins often show an association-dissociation equilibrium. Accordingly, the apparent average molar mass of such proteins closely depends on their concentration. Protein molecules with many hydrophobic spots on their surface tend to be poorly soluble.

It will be clear that temperature, pH, and salt concentration all will affect

solubility, association, and swelling of protein. The temperature especially affects hydrophobic interactions, which are weak at low and strong at high temperatures.

2.4.1.5 Denaturation

Denaturation of proteins is defined as a significant change in secondary and tertiary structures, without change in the primary structure. Examples of denaturing agents are given in Table 2.15. Denaturation per se is essentially a reversible process, but return to the native conformation on removal of the denaturing agent may be slow. Denaturation often goes along with changes in the primary structure, including changes in the —S—S— linkages (but also in other bonds; see Section 6.2). This especially occurs at high temperature and high pH, and of course by agents like mercaptoethanol. (Clearly, agent 3 in Table 2.15 does not meet the above definition of denaturation.) Furthermore, a partial *trans* → *cis* isomerization of peptide bonds involving the N-terminal side of proline residues may occur. Such secondary changes may cause denatured proteins to become insoluble and enzymes to become inactivated.

Denaturation is a cooperative change, which means that breakage of some of the bonds, occurring inside the molecule, causes the remaining bonds to be less stable, so that many of them are simultaneously broken. That explains why denaturation occurs over small ranges of temperature and concentration (see, e.g., Fig. 2.19).

2.4.1.6 Proteolysis

The peptide linkages can be hydrolyzed, e.g., by strong acid or enzymes. Enzymes involved can be exopeptidases, which split off amino acids one by one from the polypeptide chain, whether from the N terminus or the C terminus. Endopeptidases and proteases cleave somewhere in the middle of the chain. All of these enzymes are more or less specific for bonds between certain amino acid residues. Proteolysis thus yields peptides and amino acids, several of which have a distinct flavor, e.g., bitter. (Proteins themselves have no flavor.) Further degradation, e.g., by microorganisms, may result in splitting off H_2S and NH_3, which may cause offensive flavors to develop.

2.4.2 Survey of Milk Proteins

About 95% of the nitrogen in milk is in the form of proteins. Milk proteins make up a complicated mixture, from which the individual pure components are hard to separate. This is partly because some of the proteins are closely related. Furthermore, the presence of genetic variants of a particular protein may cause differences in electrophoretic mobility. Usually both variants are present in the milk (if it involves one change in the molecule), and certainly in mixed milk and even

TABLE 2.15 Examples of Denaturation Reactions

Denaturing agent	Particulars	Probable cause
1. Guanidinium chloride, $C(NH_2)_3Cl$; urea, $OC(NH_2)_2$	At high concentration	Breaks H bonds, thus also hydrophobic interactions
2. Sodium dodecyl sulfate (SDS), $CH_3(CH_2)_{11}OSO_3Na_2$	At low concentration	Stronger hydrophobic bonds with the protein than the internal ones
3. Mercaptoethanol, $HS \cdot CH_2 \cdot CH_2OH$	At pH ≥ 8	Reduces —S—S— bridges to —SH groups
4. Ethanol, $CH_3 \cdot CH_2OH$	At high concentration	Dehydrates; weakens hydrophobic interactions; enhances salt bridges
5. Some salts	At high concentration	Several, may be same as 4 above; specific interactions
6. High pH		Electrostatic repulsion; —S$^-$ group very reactive; breaks —S—S— bridge
7. High temperature		Increased effect of conformational entropy

in milk from an individual cow if it is heterozygous with respect to the gene concerned. Besides, changes may occur in proteins during their separation and purification.

All the same, the protein composition is well known. This is because milk proteins have been thoroughly investigated, not only because of their great economic importance but because of the ease of obtaining them in large quantities. Therefore, they have often been used as models for research. Table 2.16 presents an overview of the milk proteins.

Casein is defined as the protein precipitating from milk near pH 4.6. Thus it is not soluble at its isoelectric pH. Casein is not a globular protein; it associates extensively and is present in milk in large aggregates, the casein micelles, that also contain the so-called colloidal calcium phosphate (CCP). On acidification the CCP dissolves. The amino acid composition is shown in Table 2.17.

Electrophoresis reveals casein to be made up of several components (Table 2.16). The main molecular components are α_{s1}-, α_{s2}-, β-, and κ-casein. Most of the κ-casein molecules are glycosylated. α_s- and β-caseins are phosphoproteins that have phosphate groups esterified to serine; they precipitate with Ca^{2+} ions, but κ-casein ''protects'' them from precipitation, i.e., its presence in the micelles makes these stable. κ-Casein is, however, easily attacked by the rennet enzyme chymosin, which splits off a portion of the κ-casein molecule; it thereby loses its protective colloid property. As a result, the whole casein precipitates with Ca^{2+}; this reaction is the basis of the curdling of milk by rennet and thus of cheese making. Casein altered in this way is called paracasein. It thus can be obtained by means of clotting or renneting. The resulting rennet casein has a high content of calcium phosphate. (*Note*: Casein and paracasein are chemical names; acid casein and rennet casein are names of commercial products.)

Casein is hardly heat-sensitive. Only heating at temperatures above about 120°C causes the casein to slowly become insoluble. Lowering the pH of milk considerably diminishes heat stability.

Serum proteins are present in a dissolved form in the serum. They are often called whey proteins, although they are not precisely identical to the proteins of rennet whey, which also contains the peptides split off from κ-casein. The immunoglobulins in milk vary widely in concentration and composition (colostrum has a high immunoglobulin content). All serum proteins except proteosepeptone are globular proteins. At their p*I* they remain in solution, but they are heat-sensitive.

Miscellaneous proteins are numerous. The membrane of the fat globule contains several of these, including various glycoproteins; some have cysteine residues that easily generate H_2S on heating. Accordingly, H_2S is abundantly released on heating of cream. Most of the many enzyme proteins present in milk are located in the fat globule membrane. All membrane proteins also occur in the plasma, albeit in very small concentration.

TABLE 2.16   Proteins in Milk[1]

Protein	mmol/m³ milk	g/kg milk	g/100 g protein	Molar mass	g protein/ g N	Remarks
Casein	1170	26	78.5		6.36	pI ≈ 4.6
α_{s1}-Casein	440	10.0	31	~23600		Phosphoprotein
α_{S2}-Casein	110	2.6	8	~25200		Same, contains —S—S—
β-Casein	400	9.3	28	23983		Phosphoprotein
κ-Casein	180	3.3	10	~19550		"Glycoprotein"
γ-Casein	40	0.8	2.4	~20500		Part of β-casein
Serum proteins	~320	6.3	19			Soluble at pI
β-Lactoglobulin	180	3.2	9.8	18283	6.29	Contains cysteine
α-Lactalbumin	90	1.2	3.7	14176	6.25	Part of lactose synthase
Serum albumin	6	0.4	1.2	66267	6.07	
Proteose-peptone	~40	0.8	2.4	4000–40000	6.54	Heterogeneous
Immunoglobulins	~4	0.8	2.4		~6.20	Glycoproteins
IgG1, IgG2		0.65	1.8	~150000		Several types
IgA		0.14	0.4	~385000		
IgM		0.05	0.2	~900000		Part is cryoglobulin
Miscellaneous		0.8	2.5			
Lactoferrin	~1	0.1		86000		Glycoprotein, binds Fe
Transferrin	~1	0.1		76000		Glycoprotein, binds Fe
Membrane proteins		0.6	2			Glycoproteins, etc.
Enzymes						

[1] Approximate composition. pI = isoelectric pH.

TABLE 2.17 Amino Acid Composition of Some Milk Proteins (mol/kg Protein)[1]

Component	MW	Whole casein	α_s1-Casein (B)	α_s2-Casein (A)	β-Casein (A²)	κ-Casein (B)	α-Lactalbumin (B)	β-Lactoglobulin (B)	Serum albumin (B)
N	14.007	11.30	11.22	11.37	11.17	11.67	11.42	11.27	11.72
P	30.974	0.26	0.34	0.44	0.21	0.05	0.00	0.00	0.00
S	32.06	0.23	0.21	0.24	0.25	0.22	0.63	0.49	0.59
Glycine (Gly)	75.1	0.25	0.38	0.08	0.21	0.11	0.42	0.22	0.24
Alanine (Ala)	89.1	0.36	0.38	0.32	0.21	0.79	0.21	0.82	0.69
Valine (Val)	117.2	0.62	0.47	0.55	0.79	0.58	0.42	0.49	0.54
Leucine (Leu)	131.2	0.74	0.72	0.52	0.92	0.42	0.92	1.20	0.92
Isoleucine (Ile)	131.2	0.47	0.47	0.44	0.42	0.68	0.56	0.55	0.21
Proline (Pro)	115.1	1.02	0.72	0.40	1.46	1.05	0.14	0.44	0.42
Phenylalanine (Phe)	165.2	0.33	0.34	0.24	0.38	0.21	0.28	0.22	0.41
Tyrosine (Tyr)	181.2	0.34	0.42	0.48	0.17	0.47	0.28	0.22	0.29
Tryptophan (Trp)	204.2	0.06	0.08	0.08	0.04	0.05	0.28	0.11	0.03
Serine (Ser)	105.1	0.68	0.68	0.67	0.67	0.68	0.49	0.38	0.42
Threonine (Thr)	119.1	0.38	0.21	0.59	0.38	0.74	0.49	0.44	0.51
Cysteine (Cys)	121.2	0.02	0.00	0.08	0.00	0.11	0.56	0.27	0.53
Methionine (Met)	149.2	0.21	0.21	0.16	0.25	0.11	0.07	0.22	0.06
Arginine (Arg)	174.2	0.22	0.25	0.24	0.17	0.26	0.07	0.16	0.35
Histidine (His)	155.2	0.19	0.21	0.12	0.21	0.16	0.21	0.11	0.26
Lysine (Lys)	146.2	0.56	0.59	0.95	0.46	0.47	0.85	0.82	0.89
Asparagine (Asn)	132.1	0.31	0.34	0.52	0.21	0.42	0.56	0.27	0.18
Aspartic acid (Asp)	133.1	0.22	0.30	0.20	0.17	0.16	0.92	0.55	0.63
Glutamine (Gln)	146.1	0.74	0.59	0.67	0.83	0.74	0.42	0.49	0.29
Glutamic acid (Glu)	147.1	0.87	1.06	0.91	0.79	0.68	0.49	0.88	0.91
g protein per g N[1]		6.318	6.363	6.276	6.392	6.119	6.250	6.336	6.090
MW protein[1]		23192	23618	25231	23986	19026	14181	18282	66277

[1] Calculated from the amino acid sequence of each protein, including (organic) phosphate but not carbohydrate groups.

In milk, traces of various iron-binding proteins are found—in the serum, associated with the casein micelles, and in the fat globule membrane. Lactoferrin has been best examined. If pH is not too low it binds 0.12% iron (Fe^{3+}), and then has a red color.

2.4.3   Serum Proteins

Most serum proteins typically are globular proteins: they have relatively high hydrophobicity and compactly folded peptide chains. Most contain an appreciable proportion of α helix; the charge distribution is rather homogeneous (see Table 2.18). They become insoluble if milk is heated. No doubt, this change is related to, and may be caused by, denaturation of the proteins involved. To be sure, the reaction is far more complicated (see Section 6.2). The denaturation does not result in flocculation, but the proteins precipitate onto the casein micelles and remain dispersed. Colostrum, which has a very high content of serum proteins, gels when it is heated in a way comparable to the white of an egg.

α-*Lactalbumin*. In chemical terms α-lactalbumin is similar to lysozyme, but it has no bactericidal effect. Its biological function is coenzyme in the synthesis of lactose. The protein is a compactly folded, more or less spherical molecule, slightly pH- and salt-dependent. It does not associate, except at low ionic strength.

β-*Lactoglobulin* is very hydrophobic (average hydrophobicity = 5.1) as is casein, but it contains no ester-bound phosphate and fairly little proline. It has only two —S—S— linkages, and one free sulfhydryl group, which is very reactive. Its solubility closely depends on pH and ionic strength, but it does not precipitate on acidification of milk. β-Lactoglobulin is not soluble in pure water. In milk, it is present as a dimer (hence, MW = 36 566). Both molecules are tightly bound to each other, mainly by hydrophobic interactions. The dimer dissociates at high temperature. At lower pH, β-lactoglobulin associates to form an octamer. The genetic variants associate each to a different extent, i.e., A > B > C.

(Blood) serum albumin is a large molecule with many —S—S— linkages and much α helix. It is an elongated molecule, about 3×12 nm in size. Presumably, it gains entrance to milk by ''leakage'' from blood serum.

Immunoglobulins are antibodies synthesized in response to stimulation by specific antigens. They specifically occur in blood. Immunoglobulins are large protein molecules of heterogeneous composition, even within one subclass. This is no surprise, considering that they are formed by different secretory cells that each may produce different peptide chains. Moreover, a portion of the molecule is specifically formed to neutralize a particular antigen; this is the so-called hypervariable portion.

Various classes of immunoglobulins are distinguished, including G (gammaglobulins), A and M (macroglobulins), all of which occur in milk (Fig. 2.17). Each IgG molecule is a polymer of two heavy (H) and two light (L) chains;

TABLE 2.18 Properties of Some Milk Proteins

Property	α_{s1}-Casein (B)	α_{s2}-Casein (A)	β-Casein (A²)	κ-Casein (A)	β-Lactoglobulin (B)	α-Lactalbumin (B)	Serum albumin
Molar mass	23 614	25 230	23 983	19 023[1]	18 283	14 176	66 267
Amino acid residues/molecule	199	207	209	169	162	123	582
Phosphoserine (res./mol.)	8	11	5	1	0	0	0
Cysteine (res./mol.)	0	2	0	2	5	8	35
—S—S— linkages/mol.	0	1	0	—	2	4	17
Hexoses (res./mol.)	0	0	0	~2.3[2]	0[3]	0[4]	0
Hydrophobicity[5] (kJ/res.)	4.9	4.7	5.6	5.1	5.1	4.7	4.3
α-Helix (approximate %)	5–10	?	10	?	11	30	46
Charged residues (mol %)	34	36	23	21	30	28	34
Net charge/residue	−0.10	−0.07	−0.06	−0.02[2]	−0.04	−0.02	−0.02
Distribution of charge	Uneven	Uneven	Very uneven	Very uneven	Even	Even	
Isoelectric pH	4.1?	?	~5	4.1?	5.2	~4.3	4.7
Association tendency	Strong	Strong	f(T)[6]	Strong	Dimer	No	No
Ca²⁺ binding	++	++	+	—	—	([7])	—

[1] Exclusive of carbohydrate residues.
[2] Average.
[3] 8 in a rare variant (Dr).
[4] A small fraction of the molecules has carbohydrate residues.
[5] Tanford-Bigelow scale.
[6] Poor below 5°C, strong (micelle formation) at 37°C.
[7] Binds 1 mole Ca²⁺ per mole; very strong bond.
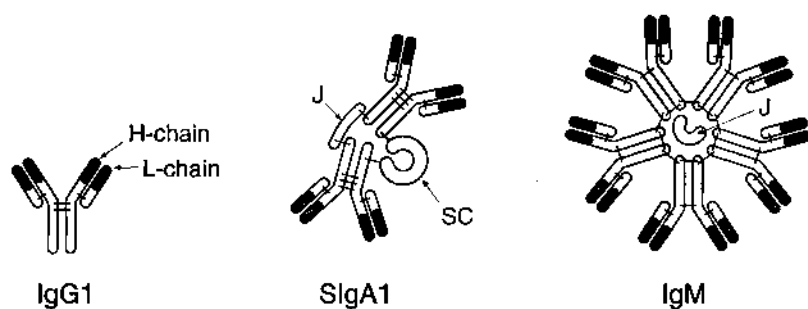
FIGURE 2.17 Schematic shape of immunoglobulins G1, secretory A1, and M. Disulfide linkages are designated by dashes. Variable portions are hatched. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

MW $\approx$ 150 000. The molecule has two identical reactive sites or junctures. The antigen involved, or often a small part of it, fits exactly into these sites. It is bound by means of several interactions: H bonds, hydrophobic bonds, electrostatic attraction. The distance between both reactive sites is flexible. This facilitates adhesion onto the antigen. The IgG immunoglobulin can exert action against many antigens and may inhibit bacterial growth. IgM consists of a pentamer of IgG-like molecules joined by a so-called J component (Fig. 2.17). It is a large molecule, with a molar mass about 900 000 and a diameter about 30 nm. IgM can be an antibody against polysaccharide groups (occur in bacterial cell wall), and especially acts against ''particles'' including bacteria and viruses. It can flocculate the ''particles'' because a single IgM molecule becomes attached to two of these (the reactive sites are on the outside of the molecule). This flocculation is called agglutination, and the IgM concerned an agglutinin. The agglutination reaction is specific with respect to the antigen, but also depends on factors like pH and ionic strength; generally, optimum pH = 5.5–7, optimum ionic strength $\approx$ 0.05. Some agglutinins exhibit cryoprecipitation, i.e., they precipitate at low temperature (<37°C, better <15°C). In doing so they can also agglutinate other particles; this is a partly nonspecific flocculation, but for the rest similar to agglutination. The proteins involved are called cryoglobulins.

In milk, IgG (1 and 2), IgA, and IgM all are present (Table 2.16). The concentrations are highly variable. High concentrations occur in colostrum, whereas very little is present in late lactation milk; but there are also significant variations among individual cows. The immunoglobulin fraction of milk may also include a lipoprotein. Little is known about the action of IgG (which has the highest concentration of the various classes) and IgA in milk; some propionic acid bacteria are inhibited by one or the other, or by both of these. However, IgM is of much importance in milk. It includes the so-called lactenins L_1 and L_3,

which are inhibitors of gram-positive bacteria. These lactenins are agglutinins. L_1 especially acts against some strains of *Streptococcus pyogenes*, L_3 against some strains of *Lactococcus lactis*. Their agglutinative action is highly specific; often, there are sensitive as well as nonsensitive bacteria in one strain. Naturally, the specific action will depend on the antigens (in this case bacteria) that the cow has encountered.

IgM includes at least one cryoglobulin. The latter is involved in the flocculation of milk fat globules (Section 3.1), which is a nonspecific reaction: each cryoglobulin present flocculates fat globules of all kinds of milk. Bacteria are also ''agglutinated'' onto fat globules. Presumably, this is a specific reaction. All these reactions cause removal of bacteria from the bulk of the milk. Common agglutination will sediment the bacteria to the bottom of the vessel. If they are agglutinated onto the fat globules, they accumulate in the cream layer. As a result, growth and action of the bacteria involved can be significantly inhibited.

The agglutinins are inactivated by heat treatment (Section 6.3). The inactivation reaction is coincident with the immunoglobulins becoming insoluble. Homogenization also inactivates the agglutinins, but the explanation is uncertain.

The main natural function of the immunoglobulins is to immunize the calf. During the first few days after parturition, the calf can absorb intact immunoglobulins from colostrum into the blood through its gastrointestinal tract. Colostrum does contain a component (a globulin-like protein) inhibiting proteolytic enzymes, trypsin in particular. In milk, this component is virtually absent. Moreover, chymosin, which does not attack immunoglobulins, occurs as a proteolytic enzyme in the abomasum of the newborn calf, rather than pepsin. As the calf grows older, ever more pepsin is produced.

Proteose-peptone is defined as non-heat-sensitive, not precipitated at pH 4.6, and precipitated by 12% trichloroacetic acid. This fraction is quite different from the other serum proteins. Three different degradation products of β-casein (the complement of the γ-caseins) largely account for the fraction. There is also a protein, i.e., a glycoprotein, that is related to a fat globule membrane constituent, and presumably there are traces of other proteins. Clearly, at neutral pH a considerable part of the proteose-peptone is present in the casein micelles, so that rennet cheese whey by no means contains all of the proteose-peptone, but serum obtained upon acidification of milk does.

Lysozyme (EC 3.2.1.17) is an enzyme that attacks polysaccharides of bacterial cell walls (splitting off muramic acid) and thereby causes partial dissolution of the cell envelope of bacteria, often resulting in lysis. Its concentration in bovine milk ($0–2$ mg·L^{-1}) usually is too low to be effective; human milk contains far more.

Lactoferrin (Table 2.16) is an inhibitor of some bacteria including *Bacillus stearothermophilus* and *B. subtilis*. The inhibition is caused by removal of iron ions from the serum. To be sure, the lactoferrin concentration in cows' milk is low; in human milk it is far higher.

2.4.4   Casein

The properties of the caseins differ from those of most proteins (Table 2.18, Fig. 2.18). Caseins are hydrophobic; they have a fairly high charge, many prolines, and few cystine residues. They do not form anything more than short lengths of α helix and have little tertiary structure. This does not imply that the casein molecules are random coils, though in dilute solution the chains are partly unfolded. Many hydrophobic groups are exposed, so that the molecules readily form hydrophobic bonds. The caseins thus show extensive association, both self-association and association with each other. (Association in casein micelles is discussed in Section 3.2.) The relatively high charge is needed to keep casein in solution.

Casein molecules cannot or can hardly be denatured because they have little secondary and tertiary structure. An example is given in Figure 2.19. β-Lactoglobulin, typically a globular protein, shows a steep conformational change at about 4 M urea, whereas β-casein changes little. Because of this, casein does not become insoluble by heating at temperatures below 100°C; it does at higher temperature, but then many reactions occur (Section 6.2).

The high charge of casein is partly caused by the phosphate groups. These are for the most part esterified to serine residues; near the pH of milk they are largely ionized (Table 2.14). The groups strongly bind divalent ions like Ca^{2+}, especially at higher pH. Figure 2.20 shows that the Ca binding parallels the content of these groups. Because of this, α_{s1}- and β-casein precipitate at fairly low calcium ion activity.

Several different caseins occur in milk, but their separation is not easy. Reactions that cause their precipitation from milk (acidification, renneting, centrifugation after adding calcium) all yield a more or less complete mixture of caseins. It was only after electrophoresis came into use that resolution of the caseins was feasible, at first into the three components α, β, and γ. Later on, α-casein could be separated into a fraction sensitive to Ca^{2+} ($\alpha_s = \alpha$-sensitive) and a Ca^{2+}-insensitive fraction, i.e., κ. Still later, further separation turned out to be necessary to obtain pure components. Currently, the complete primary structures are known. This has revealed that there are four different peptide chains—α_{s1}, α_{s2}, β, and κ, of which the molar ratio is about 4 : 1 : 4 : 1.6. Differences in phosphorylation and glycosylation, as well as some proteolysis, cause additional heterogeneity.

2.4.4.1 α_{s1}-Casein

α_{s1}-Casein has the highest charge and the highest phosphate content. Figure 2.21 shows that α_{s1}-casein associates strongly, in two steps at pH 6.6 and 0.05 M ionic strength. Obviously, very low casein concentrations are needed to obtain nonassociated molecules. On the other hand, reducing the ionic strength, and thereby increasing the effective range of the electrostatic repulsion, decreases the
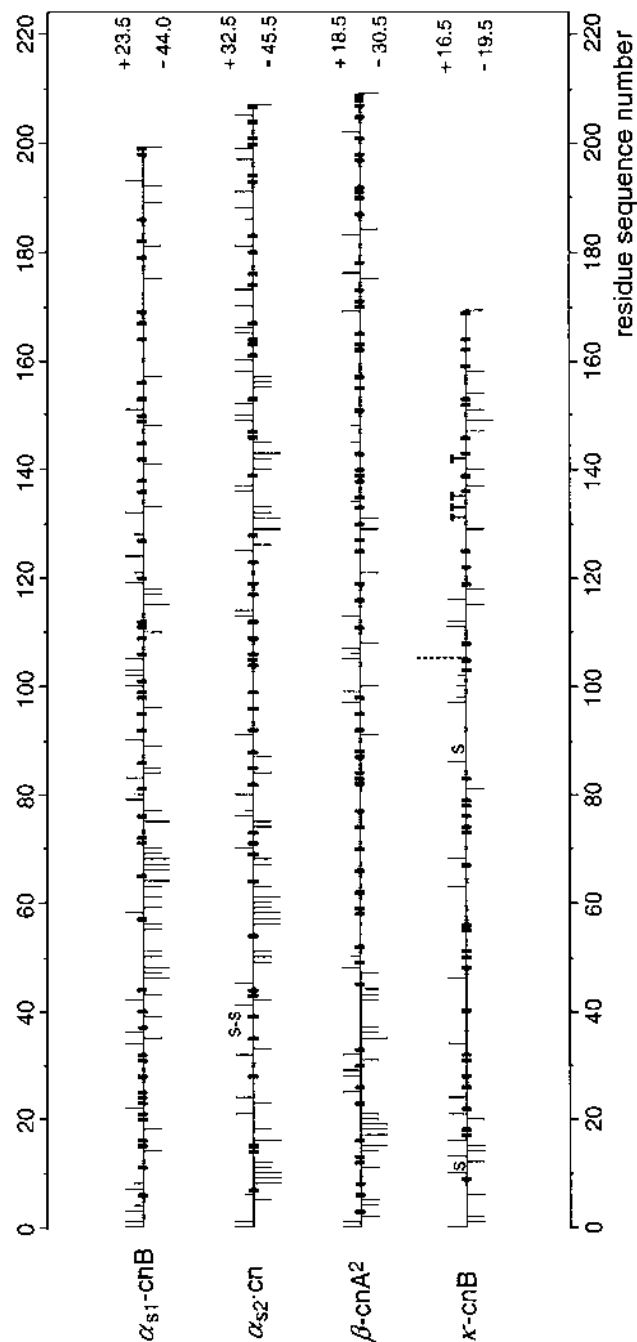
Figure 2.18 Peptide chain of caseins. The vertical bars stand for positive and negative charges, respectively (exclusive of those of neuraminic acid), where the long negative bars denote SerP and the short positive ones His. The crosses stand for proline residues, the black squares for hydrophobic amino acids, and —S—S— for cystine linkages. The broken bar indicates the point of cleavage by chymosin. Possible location of glucide residues esterified to threonine: T.

ΔE_{280}
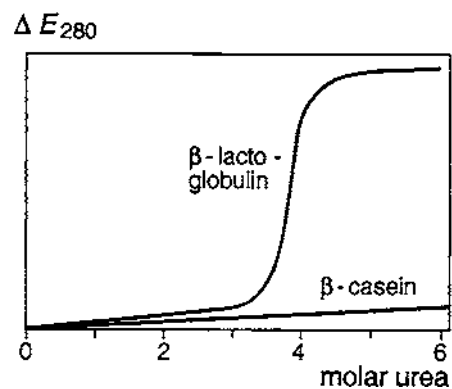
FIGURE 2.19 Change of the specific extinction at 280 nm as an indicator for denaturation by urea.

mmol Ca/g

α_{S1}; 0.38

β; 0.21

κ; 0.05

[Ca++] (mmol)
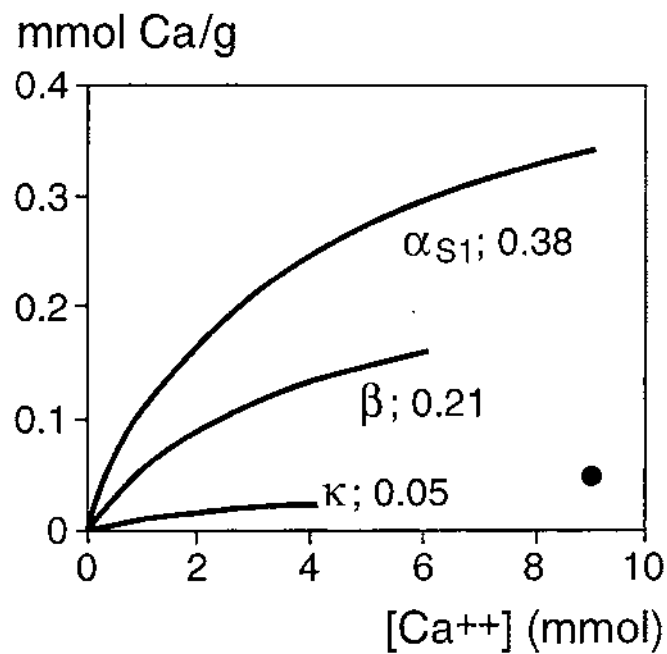
FIGURE 2.20 Binding of Ca^{2+} by caseins at pH 7.4. The ester-bound phosphate content (mmol · g^{-1} casein) is indicated. The dot refers to dephosphorylated α_{s1}-casein. After I.R. Dickson and D.J. Perkins, *Biochem. J.* **127** (1971) 235.

apparent molar mass / 10⁴
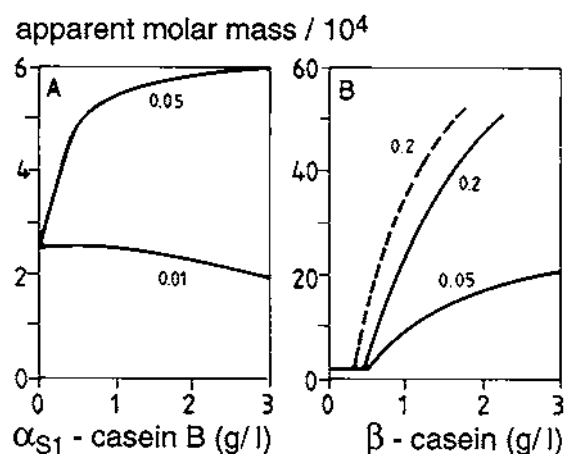
FIGURE 2.21 Association of α_{s1}- and β-casein as a function of concentration. pH 6.6 in α_{s1}-, 7.0 in β-casein. Temperature 21°C, for broken line 24°C. Ionic strength (mol · m⁻³) indicated on curves. After D.G. Schmidt and T.A.J. Payens, *Surface and Colloid Science* **9** (1976) 165.

association. Hydrophobic interactions are also involved in the association. At higher pH, i.e., greater charge, the association decreases and eventually disappears, even if casein concentration and ionic strength are high.

The variant α_{s0}-casein, which occurs in small amounts, has one more phosphate group than α_{s1}-casein.

2.4.4.2 α_{s2}-Caseins

Some variants of this protein exist. They differ by the number of ester phosphate groups, i.e., 10–14 per molecule. α_{s2}-Caseins contain two cysteine residues (forming an —S—S— bridge) and no carbohydrate groups. They are rather Ca^{2+}-sensitive.

2.4.4.3 β-Casein

β-Casein is the most hydrophobic casein and has a large number of proline residues. Moreover, as is shown in Figure 2.18, the charge is unevenly distributed. For example, dividing the molecule into two pieces, starting from the N terminus, results in the following:

Residue sequence number	1–43	44–209
Proline frequency	0.02	0.20
Charge frequency	0.65	0.12
Net charge	−15.5	+4.5
Average hydrophobicity (kJ per residue)	3.3	6.0

Obviously, both parts differ considerably in properties. Thus, β-casein is somewhat like a soap molecule with a hydrophilic, charged ''head'' and a long-chain, apolar ''tail.'' The association of β-casein is somewhat like that of a soap in that a critical micelle concentration occurs; see Figure 2.21. The micelles (here in the strict sense used by physical chemists) comprise some 20 or 30 molecules. Note the strong dependence of the association on temperature and ionic strength. Below 5°C no association of β-casein occurs and the molecule remains unfolded. Now it more or less behaves like a random coil. In milk, part of the β-casein goes into solution at low temperature, thereby increasing the viscosity of the milk. These changes are reversible but occur slowly (hours).

2.4.4.4 γ-Casein

γ-Casein is a degradation product of β-casein. It corresponds for the most part to amino acid residues 29–209 of the β-casein sequence, i.e., the more hydrophobic portion. Accordingly, it is fairly soluble in ethanol (900 mg · L^{-1} in 50% ethanol; α_s-casein, e.g., only 9 mg · L^{-1}). The cleavage is caused by the enzyme plasmin (EC 3.4.21.7), present in milk. The amount of γ-caseins can vary widely, depending on the age and the keeping temperature of the milk. The split-off parts constitute most of the proteose-peptones.

2.4.4.5 κ-Casein

κ-Casein greatly differs from the other caseins. It has two cysteine residues that may form intermolecular disulfide bonds. Because of this, κ-casein occurs in milk as oligomers containing 5–11 monomers. About two-thirds of the molecules contain a carbohydrate group, which is esterified to one of the threonines (131, 133, 135, or 142) and has galactosamine, galactose, and one or two N-acetylneuraminic acid (NANA or *o*-sialic acid) residues; see Figure 2.22. These groups each have one or two negative charges and are quite hydrophilic. Some other, minor configurations occur as well. So there are obvious differences among κ-casein molecules, also because some of them have two ester phosphate groups rather than one. This so-called microheterogeneity always occurs, even within an individual milking of one cow.

The peptide bond between residues 105 and 106 is rapidly hydrolyzed by enzymes. Note that there is a positively charged region near this site (Fig. 2.18).

κ-Casein also strongly associates to yield micelles that contain over 30 molecules including protruding carbohydrate groups. The association is somewhat like that of β-casein. Between the molecules there are great differences in association that are related to the differences in carbohydrate content.

2.4.4.6 Biological Functions of Casein

Naturally, casein should provide the calf with nutrients. But not only amino acids are involved; Ca and phosphate are important components as well. Obviously,
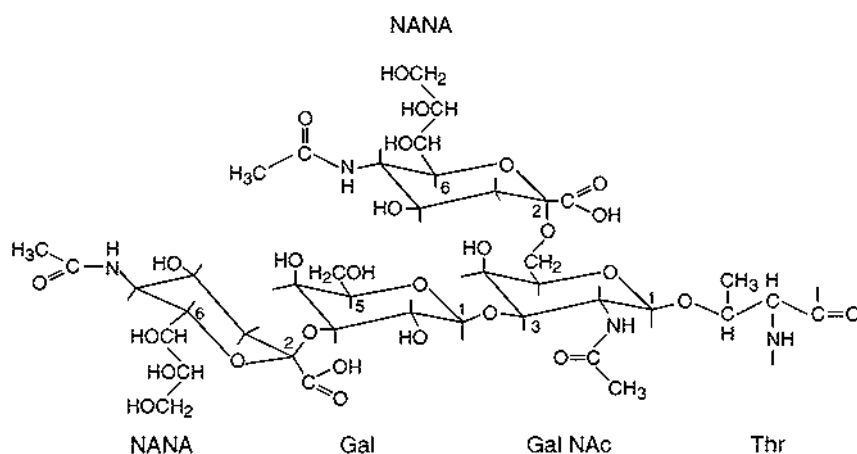
FIGURE 2.22 Example of a glucide group linked to κ-casein. Often the NANA residue at the top is lacking.

casein has evolved in such a way that it can bind large amounts of the poorly soluble calcium phosphate while keeping these substances in a stable suspension. The structure and stability of the casein micelles formed in that way are discussed further in Section 3.2.

2.5 ENZYMES

Milk contains scores of enzymes. The native enzymes, i.e., those known to be excreted by the mammary gland, may include several present in the leukocytes, e.g., catalase. In addition, enzymes of microbial origin may be involved. The latter may be present in microorganisms, secreted by the organisms (e.g., proteinases and lipases), or released after lysis. The native enzymes can be present at different locations in the milk. Many of them are associated with the fat globule membrane. This is no surprise, considering that most of the membrane originates from the apical cell membrane, which contains several enzymes. Other enzymes are in solution, i.e., dispersed in the serum, but some of these (e.g., lipoprotein lipase) are partly associated with the casein micelles.

Most of the milk enzymes seem to have no biological function in milk, even if they are present in high concentrations (e.g., ribonuclease; Table 2.19). Often, they do not significantly alter the milk. Some enzymes have an antimicrobial function or play other beneficial roles. A few of the enzymes may facilitate resorption of milk constituents into the blood if and when milking is stopped. It presumably concerns plasmin and lipoprotein lipase, which are not very active

TABLE 2.19 Some Enzymes in Milk

Name	EC number	Optimum pH	Optimum temperature (°C)	Activity[1] potential	Activity[1] actual	Where in milk?	Inactivation[2]
Xanthine oxidase	1.1.3.22	~8	37	>>40	40	Fat globule membrane	7 min 73°C
Sulfhydryl oxidase	1.8.?	~7	~45	?	?	Plasma	3 min 73°C
Catalase	1.11.1.6	7	37?	?	300	Leukocytes	2 min 73°C
Lactoperoxidase	1.11.1.7	6.5	20	?	22000	Serum	10 min 73°C
Superoxide dismutase	1.15.1.1	?	?	~2000	?	Plasma	70 min 76°C
Lipoprotein lipase	3.1.1.34	~9	33	3000	0.3	Casein micelles	30 s 73°C
Alkaline phosphatase	3.1.3.1	~9	37	500	<<500	Fat globule membrane	20 s 73°C
Ribonuclease	3.1.27.5	7.5	37	([3])	?	Serum	?
Plasmin	3.4.21.7	8	37	3	0.05	Casein micelles	40 min 73°C

[1] μmol $\cdot$ min^{-1} $\cdot$ L^{-1}.
[2] Heat treatment needed to reduce activity to approximately 1%.
[3] 11–25 mg enzyme/kg milk.

in fresh milk though they are present in high concentrations (Table 2.19). These, as well as some other enzymes, can cause spoilage of milk during storage. Some enzymes are used for analytical purposes. Formerly, catalase was applied to detect mastitis, but the correlation is too weak. N-Acetyl-β-ᴅ-glucosaminidase (EC 3.2.1.30), also called NAGase, is now considered a better marker, although again there is no perfect relation with mastitis. Furthermore, particular enzymes are used to monitor pasteurization. Some examples of native milk enzymes and their action are discussed in Section 2.5.2.

2.5.1 Enzyme Activity

The properties of a solution are governed by activities rather than by concentrations, and this certainly holds for enzymes. The maximum rate of catalysis is expressed as k_{cat} or turnover number for the enzyme, i.e., the number of molecules of substrate converted per enzyme molecule per second if the substrate is in excess and the conditions for the enzyme (pH, temperature, ionic strength, and other factors affecting enzyme activity) are ideal. The total turnover rate is defined by $V_{max} = k_{cat} [E]$, where $[E]$ = enzyme concentration. According to Michaelis and Menten, the velocity of reaction as a function of the substrate concentration $[S]$ is

$$v_i = V_{max}[S]/(K_m + [S]) \tag{2.5}$$

An example of the relationship is given in Figure 2.23. The Michaelis constant K_m is a measure of the affinity of an enzyme for its substrate (the lower K_m, the greater the affinity). It depends on the type of substrate used and is thus a variable next to k_{cat}. K_m equals the substrate concentration when $v_i = V_{max}/2$; see Figure 2.23. Equation (2.5) only applies to the initial velocity of reaction v_i because reaction products may inhibit the reaction (product inhibition); moreover, the substrate concentration decreases during the reaction.

The following are additional factors that can affect enzyme activity.

a. In addition to the above-mentioned reaction products, several substances may inhibit enzyme activity, e.g., because these substances also bind to the enzyme (competitive inhibition) or because they affect the conformation of the enzyme molecule.
b. Equation (2.5) does not apply to so-called allosteric enzymes. This aspect will not be discussed further.
c. Many enzymes need a ''cofactor.'' An example is apoprotein C_2, which is essential for lipoprotein lipase action. The concentration of cofactors in milk varies.
d. There may be other stimulators that inactivate an inhibitor.
e. The substrate can be inaccessible. An example is the triglycerides, which are screened from enzymes in milk by the fat globule membrane.
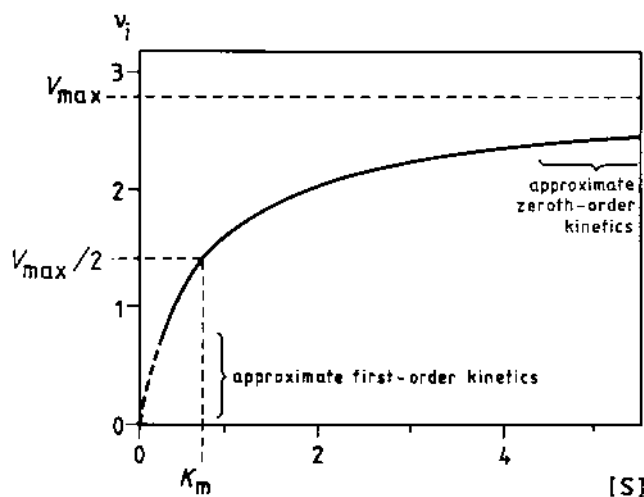
FIGURE 2.23 Effect of substrate concentration [S] on initial reaction rate v_i of an enzyme reaction, according to Eq. (2.5). Arbitrary scale.

    f. The enzyme can be adsorbed onto particles, thereby becoming less active. Some enzymes (lipases, proteinases) are adsorbed onto casein micelles.

    g. The enzyme may be present in a nonactive form, a so-called zymogen, and slowly be activated. An example is plasmin, largely occurring in milk as the inactive plasminogen.

    h. The enzyme may be (slowly) inactivated. For instance, lipoprotein lipase in milk loses activity, presumably caused by an oxidative reaction.

2.5.2 Some Milk Enzymes

2.5.2.1 Antibacterial Enzymes

The main representative is *lactoperoxidase* (EC 1.11.1.7). It catalyzes the reaction:

$$H_2O_2 + 2HA \rightarrow 2H_2O + 2A$$
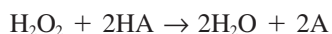
where the substrate HA can include several compounds: aromatic amines, phenols, vitamin C, and so on. The enzyme can also catalyze oxidation of thiocyanate (CNS^-) by H_2O_2 to an unidentified product that inhibits most bacteria. If the bacteria themselves produce H_2O_2, as most lactic acid bacteria do, they are inhibited. (In milk, H_2O_2 decomposition by catalase, EC 1.11.1.6, is too weak to prevent this.) But the thiocyanate concentration in milk varies widely because it

depends on the cyanoglucoside content of the feed. Sometimes thiocyanate plus a little H_2O_2 is added to raw milk to prevent spoilage. In this way, even in a warm climate spoilage of milk can be delayed for many hours. In milk, the action of the lactoperoxidase–hydrogen peroxide–thiocyanate system (which also occurs in saliva) can be enhanced by xanthine oxidase (see below), which can form H_2O_2 from some substrates.

     Lysozyme (EC 3.2.1.17) is another bactericidal enzyme; it hydrolyzes polysaccharides of bacterial cell walls, eventually causing lysis of the bacteria (see also Section 24.2). In cows' milk the lysozyme activity is weak; in human milk it is much stronger.

2.5.2.2 Oxidoreductases

Xanthine oxidase (EC 1.1.3.22) can catalyze oxidation of various substances, by no means xanthine only. Many substances, including O_2, can be a hydrogen acceptor. The enzyme can reduce nitrate (which occurs in milk only in trace quantities) to nitrite. This property is put to use in the manufacture of some cheeses, where nitrate is added to milk to prevent the proliferation of the detrimental butyric acid bacteria (Section 24.2). Nitrite inhibits these bacteria. If nitrate has been added to the milk, nitrite is present in sufficient amounts in cheese, though it is fairly rapidly decomposed. Cows' milk has a relatively high xanthine oxidase content. Most of the enzyme is associated with the fat globule membrane. Because of this it is only partly active, but the activity is increased by such treatments as cooling and homogenization, which may release enzyme from the membrane.

     Superoxide dismutase (EC 1.15.1.1) catalyzes the dismutation (dismutation here meaning oxidation of one molecule and simultaneous reduction of another) of superoxide anion O_2^- to hydrogen peroxide and triplet oxygen according to:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + {}^3O_2$$
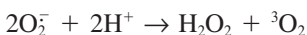
The enzyme in milk is similar if not identical to that in blood. Its biological function is to protect cells from oxidative damage. In milk, the superoxide anion can be generated by oxidations catalyzed by xanthine oxidase and lactoperoxidase, and by photooxidation of riboflavin. Superoxide dismutase may inhibit oxidation of milk constituents. The enzyme is supposed to counteract autoxidation of lipids (this oxidation causes off-flavors; see Section 2.3). It is not inactivated by low pasteurization.

     Sulfhydryl oxidase (EC 1.8.?) catalyzes oxidation of sulfhydryl groups to disulfides, using O_2 as electron acceptor:

$$2RSH + O_2 \rightarrow RSSR + H_2O_2$$
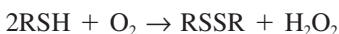
-SH groups of both high- and low-molar-mass compounds are decomposed. Most of the enzyme is bound to lipoprotein particles. Pasteurization inactivates the

enzyme only partially. In pasteurized milk, the enzyme may be essential for reducing the cooked flavor caused by SH compounds.

Lactoperoxidase (see above) is also an oxidoreductase.

2.5.2.3   Phosphatases

Several phosphatases occur in milk. Best-known is milk *alkaline phosphatase* (EC 3.1.3.1), which catalyzes the hydrolysis of phosphoric monoesters. Generally, determination of the activity of the enzyme by the ''phosphatase activity test'' is applied as a check of low pasteurization of milk. Inactivation of the enzyme ensures that all of the pathogenic microorganisms (as far as these can grow in milk) present in the milk during heating have been killed; most but not all of the lactic acid bacteria and Gram-negative rods have also been killed. Most of the enzyme is in the membranes of the fat globules. Accordingly, the phosphatase test is less sensitive when applied to skim milk.

Milk also contains an *acid phosphatase* (EC 3.1.3.2); it occurs in the serum and is quite heat-resistant (see Fig. 2.25). These phosphatases catalyze the hydrolysis of certain phosphoric esters in milk, but slowly. Another phosphatase can release phosphoric acid groups esterified to casein.

2.5.2.4   Lipolytic Enzymes

Several esterases, which can hydrolyze fatty acid esters, occur in milk. Some of these attack esters in solution, but the principal lipolytic enzyme of cows' milk, i.e., *lipoprotein lipase* (EC 3.1.1.34), liberates fatty acids from tri- and diglycerides and is only active at the oil–water interface. It is bound largely to casein micelles. In milk, lipolysis causes a soapy-rancid off-flavor and this is further discussed in Section 3.1.

2.5.2.5   Proteinases

In milk at least two trypsin-like endopeptidases occur. One of these is the so-called *alkaline milk proteinase*, which is identical to the plasmin of blood (EC 3.4.21.7). Most of the alkaline proteinase in milk is present as the inactive plasminogen. The enzyme is largely associated with the casein micelles. Its activity in milk varies widely, partly because of a variable ratio of plasmin to plasminogen. Usually, the activity increases with time as well as by heating, e.g., pasteurization. The explanation appears to be that milk contains one or more promotors that catalyze the hydrolysis of plasminogen to yield plasmin. Moreover, milk contains at least one substance that inhibits the promotor(s). The inhibitor is inactivated by heat treatment. It also appears that leukocytes contain a promotor, and milk of a high somatic cell count generally shows enhanced plasmin activity.

Plasmin can hydrolyze proteins to yield large degradation products and is responsible for production of γ-casein and proteose-peptones from β-casein. The enzyme causes proteolysis in some products, e.g., in cheese. In UHT milk
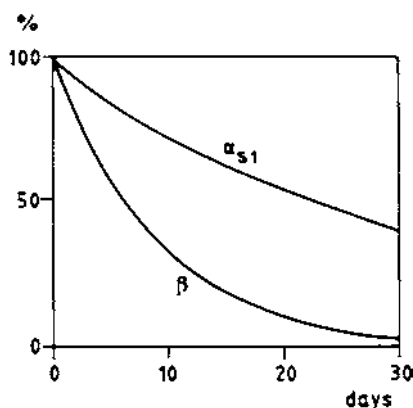
FIGURE 2.24 Plasmin action on α_{s1}- and β-casein in milk at 20°C. Casein left (in %) as a function of storage time. The milk had been heated for 5 s at 134°C. After results by F.M. Driessen (Ph.D. Thesis, Wageningen, 1983).

products (Fig. 2.24) its proteolytic action causes a bitter flavor and eventually can solubilize the casein micelles; in some cases, gelation has been observed. This is because the enzyme is very heat-resistant (Fig. 2.25). Accordingly, appropriate UHT treatment (e.g., 140°C for 15 s) should be applied to prevent such problems.

In milk an *acid milk proteinase* also occurs, though with a lower activity. The enzyme is less heat-resistant than plasmin.

2.5.3 Inactivation

To inactivate enzymes, heat treatment is mostly applied. Inactivation is generally due to denaturation (unfolding) of the enzyme molecule. The kinetics of the heat-induced reactions are discussed in Section 6.3. The heating time–temperature relationship for the inactivation of various enzymes in milk is given in Figure 2.25. Inactivation by heat treatment is of great importance because

a. Enzymes that cause spoilage can be inactivated.
b. Systems that inhibit bacterial growth can be inactivated. This can be either desirable or undesirable.
c. Spoilage inhibiting enzymes (e.g., superoxide dismutase) can be inactivated.
d. The intensity of milk pasteurization can be checked, low pasteurization through alkaline phosphatase and high pasteurization through lactoperoxidase.
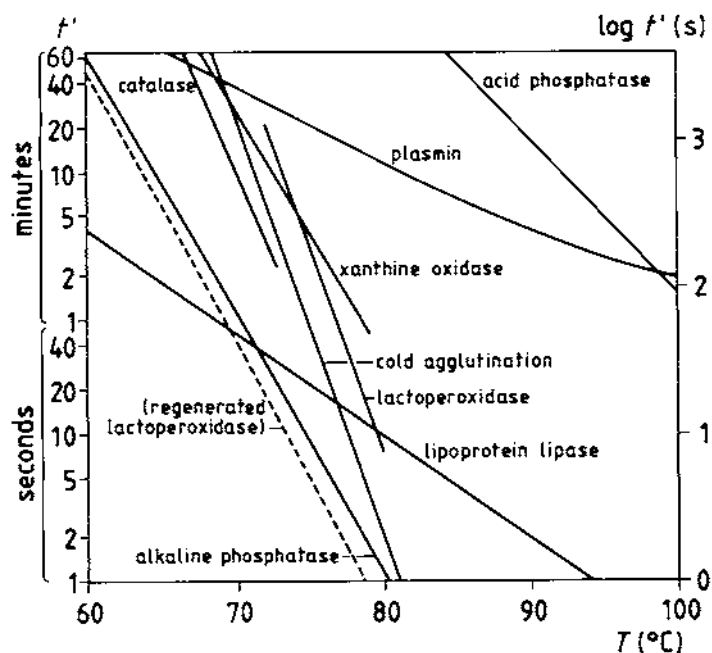
FIGURE 2.25   Time (t') and temperature (T) of heating milk needed to inactivate some enzymes (i.e., reduce the activity by about 99%) and to prevent cold agglutination. Approximate examples. After P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

The rate of inactivation by heat treatment may closely depend on conditions like pH and the presence or absence of substrate. Moreover, different isozymes (i.e., genetic variants of one enzyme) that differ in heat stability may be involved. Often, they cause curved plots for the relationship between the log of the required heating time and the temperature.

Another complication is reactivation, which means that the heat-inactivated enzyme returns to the active form after cooling. Many enzymes do not exhibit reactivation, whereas other enzymes slowly do so. Examples are alkaline phosphatase, which becomes partly reactivated a few days after heat treatment, especially in cream (because of its high phosphatase content), and lactoperoxidase. Reactivated lactoperoxidase is also heat-labile, even more than the original enzyme (Fig. 2.25). Some enzymes are reactivated very rapidly after cooling. It then seems as if the heat treatment has had hardly any effect on the enzyme activity. On the other hand, heating may have been so intense that the unfolded enzyme molecule has reacted such that reactivation of the enzyme is not possible

anymore. The inactivation plots then usually are curved and not very steep, and high temperatures and long times are needed. An example in Figure 2.25 is plasmin.

Alternatively, enzymes can be inactivated by oxidation processes, sometimes by strong and prolonged shearing, by excessive aeration, etc. Homogenization rarely has a significant effect.

It can be further noted that a low enzyme activity observed after a certain treatment or under certain conditions does not necessarily mean that part of the enzyme has been inactivated. It may be that only the specific activity of the enzyme, i.e., k_{cat}, is low. That can be due to unfavorable conditions (pH, temperature, ionic strength, etc.) or to the presence of inhibitors.

2.6 OTHER COMPONENTS

Sections 2.1–2.4 deal with the main components in milk (with the exception of water, which actually is the main component). Several minor components are also present, but these are not necessarily unimportant in all respects. The number of compounds detected in milk is large and will certainly increase with further research and with improvements in the sensitivity of analytical methods. It is often uncertain as to whether such a component occurs as such or is chemically bound. Sometimes components alter during separation and analysis.

The ''other'' components are partly ''natural''; they partially gain access to milk by contamination, partially by enzymatic and microbial changes, or by changes caused by manufacturing processes. A component, though natural in milk, may considerably increase in concentration due to contamination, etc.

2.6.1 Natural Components

The reader is referred to Table 1.3. Most of these components stem directly from the blood or are intermediate products of the metabolic processes in the secretory cell. Some groups of components will be considered here. They are grouped arbitrarily and several components belong to more than one category.

a. *Organic acids*. In addition to citric acid (Section 2.2) and low-molar-mass fatty acids (Section 2.3), small quantities of other organic acids (e.g., trace amounts of lactic and pyruvic acid) occur in milk serum. Bacterial action may greatly increase the concentration of such acids.
b. *Carbohydrates*. In addition to lactose, milk contains traces of glucose, galactose, and oligosaccharides. Furthermore, it contains several sugar derivatives, again in trace quantities: hexose monophosphates, hexosamines, and many others. A large part of these is associated with other compounds, e.g., proteins (κ-casein, fat globule membrane proteins) and cerebrosides.

c.   *Nitrogenous compounds*. On average, about 5% of total nitrogen in milk is nonprotein nitrogen (NPN). Groups of NPN compounds are listed in Table 2.20. The compounds are partly intermediate products of the protein metabolism of the animal (e.g., ammonia, urea, creatine, creatinine, uric acid). Most of the amino acids as well as their derivatives (amines, serine phosphoric acid) are also found in trace amounts free in solution. Milk also contains small peptides. These compounds may be essential nutrients for some bacteria.

d.   *Vitamins*. All known vitamins are present in milk.

e.   *Phosphate esters*. See also Section 2.2. Examples are hexose phosphates and glycerol phosphate.

f.   *Ribonucleic acids and their degradation products*, e.g., phosphate esters and organic bases. Furthermore, orotic acid typically occurs in milk of ruminant animals; it is a growth factor for *Lactobacillus delbrueckii* ssp. *bulgaricus*.

g.   *Sulfuric acid esters*. Only indoxyl sulfate has been found in milk.

h.   *Carbonyl compounds*. An example is acetone; more of it occurs if the cow suffers from ketosis. Fat-soluble aldehydes and ketones are mentioned in Section 2.3.2.

i.   *Several lipids*. See Section 2.3.2.

j.   *Trace elements*. See Section 2.2.

k.   *Gases*. In milk the amount of nitrogen is about 16 mg · kg^{-1}, that of oxygen about 6 mg · kg^{-1}, or about 1.3% and 0.4% by volume, respectively. Milk is almost saturated with respect to air; however,

TABLE 2.20   Nonprotein Nitrogenous Compounds in Milk[1]

Compound	Conc. (mg · kg^{-1})
Urea-N	84–280
Creatine-N	6–20
Creatinine-N	2–9
Uric acid-N	5–8
Orotic acid-N	4–30
Hippuric acid-N	4
Peptide-N	~30[2]
Ammonia-N	3–14
α-Amino-acid-N	29–51
Total NPN	229–308

[1] Approximate range of contents reported.
[2] Greatly depends on analytical method.

it contains relatively far more carbon dioxide though in the form of bicarbonate (Section 2.2). The O_2 content of milk while in the udder is lower, i.e., about 1.5 mg · kg^{-1}.

l. *Enzymes*. See Section 2.5.
m. *Hormones*. Several hormones are present in trace quantities in milk. Examples are prolactin, somatotropin, and steroids.
n. *Somatic cells*, together with all compounds they contain (Section 1.1.2).

In addition to the compounds mentioned above, milk contains numerous others. For instance, it contains about 3 mg ethanol per kg (per year, a cow produces an amount of ethanol sufficient for a glass of wine).

2.6.2   Contaminants

In principle, the number of compounds that can enter milk by contamination is endless. There is much concern about compounds that may be harmful to the consumer because of their potential toxicity or mutagenicity; furthermore, some people may be allergic to compounds, e.g., antibiotics. It has hardly ever happened that a compound, though harmful in principle, was found in such concentration in milk that it would produce a health hazard. Furthermore, investigations have concentrated on contaminants that cause undesirable effects during manufacture or storage of milk or milk products.

There are several pathways by which contaminants can gain entrance into milk; some are known to enter milk in more than one way.

a. *Illness of the cow*. For example, severe mastitis causes blood compounds and somatic cells to enter the milk (Section 1.3.1.7).
b. *Pharmaceuticals* (drugs) that have been administered to the cow. Antibiotics are widely used; they are introduced into the udder to treat mastitis and can still be detected in milk 3 or 4 days after they have been administered. Antibiotics in milk may slow down the action of lactic acid bacteria. Several pharmaceuticals can enter the milk through the blood.
c. *Feed*. Many compounds can gain entrance into the milk through the feed, though the cow may act as a filter. Sometimes, substances are partly broken down first. The following list gives examples.
   Chlorinated hydrocarbons, such as several pesticides (DDT, aldrin, dieldrin); PCBs (polychlorinated biphenyls), which are widely used in materials; dioxins, which are potentially harmful to the consumer even in extremely low concentrations. Some of these components are toxic or carcinogenic, and occasionally too high a level (i.e., higher than the standard, which normally has a safety factor of, say,

100) has been detected, for instance, in milk from cows fed large quantities of vegetables sprayed with pesticides. These substances are lipophilic and hence tend to accumulate in the fat.

Other pesticides, herbicides, and fungicides like phosphoric esters and carbamates. Most of these components are broken down by the cow.

Mycotoxins may originate from molds growing on concentrates fed to cows. Particularly suspect are the harmful aflatoxins. In many countries, the feed has to comply with strict requirements.

Heavy metals. Pb, Hg, and Cd are especially suspect, but toxic levels have never been found in milk. Most heavy metals do not gain entrance into the milk because the cow acts as a filter, unless extremely high quantities are fed.

Radionuclides. See Section 2.6.3.

d. The following are examples of compounds that may enter the milk during milking and milk handling.

Pesticides. These can also gain entrance into the milk through the air, e.g., when aerosols with insecticides are used.

Plasticizers from plastics or antioxidants from rubber (teat cup lining).

Metal ions, especially Cu. These may cause off-flavors via autoxidation.

Cleaning agents and disinfectants. These may cause off-flavors and decreased activity of starters.

e. Substances added on purpose. Sometimes disinfectants are added to milk to arrive at a low colony count. This is, of course, adulteration. Active chlorine may be determined to detect such adulteration. Addition of water is best checked by determining the freezing point.

2.6.3 Radionuclides

Radioactive isotopes of several elements are always present in milk but in minute quantities. It especially concerns ^{40}K. If feed or drinking water contaminated after radioactive fallout is ingested by the cow, part of the radionuclides will be secreted in the milk, despite the fact that the cow acts as a filter. For example, of the radioactive Sr ingested only a small part enters the milk, of ^{131}I much more.

Table 2.21 lists radionuclides that are the most harmful to the consumer and that may enter milk, with some particulars. ^{90}Sr is of great concern because the physical as well as the biological half-life times are long. The physical half-life refers to the period needed to reduce the radioactive emission by an isotope to half of its original level. The biological half-life refers to the time it takes until half of the amount of a compound ingested by the body has been excreted. The latter depends closely on conditions. Table 2.21 refers to the half-life of the most tenaciously held pool of the element; a large part of it is often excreted more

TABLE 2.21 Most Important Radionuclides That Can Occur in Milk

Radionuclide	Physical half-life	Biological half-life	Location in milk
^{89}Sr	52 days	~50 years	>80% in casein micelles, the rest in serum
^{90}Sr	28 years	~50 years	>80% in casein micelles, the rest in serum
^{131}I	8 days	~100 days	Serum (~2% in the fat)
^{137}Cs	33 years	~30 days	Serum

quickly. Furthermore, if appreciable accumulation of ^{90}Sr in the bone is to be prevented, it is a low ratio of ^{90}Sr to Ca that is important rather than a small quantity of ^{90}Sr taken up. ^{131}I is considered to be particularly hazardous for fairly short periods after serious fallout. Iodine accumulates in the thyroid gland. Atomic bomb testing especially leads to emission of radioactive Sr and I. Nuclear reactor accidents can cause fairly serious contamination by ^{137}Cs.

Strontium is distributed in milk in much the same way as Ca, but because $SrHPO_4$ is very poorly soluble, by far the greater part of the Sr in milk is in the colloidal phosphate. Cs behaves like K^+ and Na^+. Most iodine is found as dissolved iodide.

2.6.4 Flavor Compounds

The main flavor compounds in milk are lactose and the dissolved salts, which cause a sweet and salty taste, respectively. The sweet taste prevails, whereas the salty taste is prevalent if the Cl/lactose ratio is high, as in mastitic milk. The fat globules must also contain flavor compounds since skim milk and milk differ considerably in flavor; these are responsible for the creamy or ''rich'' flavor, though milk with an increased fat-free dry matter content also has enhanced ''richness.'' Some flavor compounds from the fat are discussed in Section 2.3.

Other compounds, including dimethyl sulfide, diacetyl, 2-methylbutanol-1, and some aldehydes, are responsible for the characteristic flavor of fresh raw milk. All in all, milk has a little pronounced flavor, which implies that defects occur readily. The literature concerned may be confusing. This is because off-flavors are hard to specify and are subjectively judged. Often the substances causing mild off-flavors are not well known.

Fresh milk may have off-flavors originating from the feed. The compounds

causing the off-flavors enter the milk through the cow, the air, and sometimes through both pathways. Examples are clover and garlic flavors. If the cow suffers from ketosis, such as that caused by feed deficient in protein, increased concentrations of ketones (including acetone) are found in the milk. Consequently, the milk exhibits a typical cowy flavor. Vacuum heating may remove part of such flavor compounds if they are hydrophilic. Removal of the many fat-soluble compounds is much more difficult. Hay feeding leads to cumarin in milk; this flavor compound is not always undesirable.

Spoilage of milk, especially microbial spoilage, may produce flavor defects; the defects are referred to as acid, unclean, fruity/ester, malty/burnt, phenolic, bitter, rancid flavors, etc. Enzymatic spoilage includes lipolysis (Section 3.1). Autoxidation of fat, as caused by catalytic action of Cu, usually leads to a tallowy flavor (Section 2.3). In milk, a cardboard flavor also occurs, which results from autoxidation of phospholipids; it can be observed in skim milk as well. The phospholipids in the plasma appear to oxidize more readily. In sour-cream buttermilk, this may readily lead to a metallic flavor if the defect is weak and to a sharp (pungent) flavor if the defect is strong.

Flavor defects in milk can also be induced by light. Often direct sunlight applied for 10 min, or diffused natural light for some longer time, or light from fluorescent lamps suffices. The tallowy flavors involved do not develop immediately, but only after some time. They may simply result from autoxidation catalyzed by light. But on exposure to light, additional flavor compounds can also develop in milk. Development of this ''sunlight flavor'' requires riboflavin (vitamin B_2). Oxidation of free methionine yields methional ($CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot CHO$), whereas free SH compounds are formed from protein-associated sulfur-containing amino acids. Presumably, all of these compounds together cause the sunlight flavor.

Moderate heat treatment of milk (say, 75°C for 20 s) causes the characteristic raw milk flavor to disappear so that a fairly flat flavor results. More intense heat treatment, e.g., 80–100°C for 20 s, results in ''cooked flavor,'' caused mainly by H_2S. The preponderant ''sterilization flavor'' of high-heated milk (e.g., 115°C for 10 min) mainly results from maltol, furanone compounds (formed from lactose), and aliphatic methyl ketones and lactones (formed from lipids).

SUGGESTED LITERATURE

- In addition to the textbook mentioned at the end of Chapter 1, the following reference books contain extensive information on the chemistry and physical aspects of milk components.

  P.F. Fox, ed., *Advanced Dairy Chemistry*, which is available in three volumes:

  1. *Proteins*, Elsevier, London, 1992.

2. *Lipids*, 2nd ed., Chapman and Hall, London, 1995.

3. *Lactose, Water, Salts and Vitamins*, 2nd ed., Chapman and Hall, London, 1997.

(Volume 1 also includes indigenous milk enzymes.)

and

N.P. Wong, ed., *Fundamentals of Dairy Chemistry*, 3rd ed., Van Nostrand Reinhold, New York, 1988.

- Basic aspects of the chemistry of milk components are to be found in texts on organic chemistry and biochemistry, and also in:

O.R. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996.

- Contaminants of milk are discussed in:

Monograph on Residues and Contaminants in Milk and Milk Products, International Dairy Federation, special issue, Brussels, 1991.

- Trace elements in milk are also in an IDF report:

Trace elements in milk and milk products, *Bulletin of the International Dairy Federation, 278*, Brussels, 1992.

3

Colloidal Particles of Milk

3.1 FAT GLOBULES

Nearly all of the fat in milk is in separate small globules. Milk is thus an oil-in-water emulsion. The physicochemical aspects of the emulsion are essential, especially when considering the changes that occur during storage and processing of milk.

3.1.1 Properties

3.1.1.1 Size Distribution

The milk fat globules vary in diameter from about 0.1 to 15 μm and are thus characterized by a size distribution. If, in size class i, N_i is the number of particles with diameter d_i (or, more precisely, with diameters between $d_i + \frac{1}{2}\Delta d$ and $d_i - \frac{1}{2}\Delta d$) per unit volume of milk, the number frequency is given by $N_i/\Delta d$ (dimension $[L^{-4}]$). The volume frequency, which gives the amount of fat present in globules of a certain diameter, is found from $\pi N_i \cdot d_i^3/6\Delta d$ (dimension $[L^{-1}]$). We may also express the volume frequency as percentage of the total volume of fat per unit class width. It then is almost equal to the mass frequency. Examples are given in Figure 3.1; it is seen that milk contains very many small globules, which comprise only a small fraction of the total fat.

Several properties of a dispersion are affected by the size distribution. Table 3.1 gives some parameters of size distributions that are essential to these properties. The number average diameter $\bar{d}$ is not a very useful parameter since the total number of fat globules S_0 is so large and difficult to obtain: the many small globules can hardly be counted. Diameter d_{vs} is more useful. It relates the volume
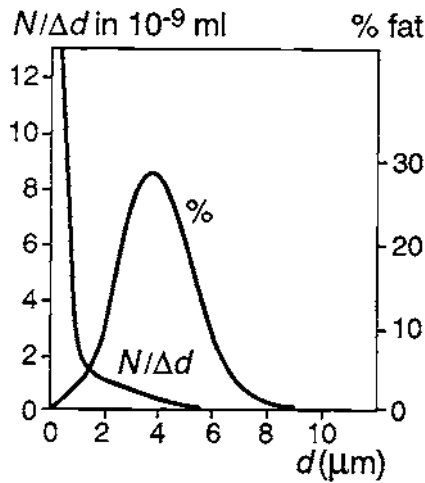
FIGURE 3.1   Average size frequency distribution of the fat globules in milk of Friesian cows (d_{vs} = 3.4 μm, fat content 3.9%). Number ($N/\Delta d$) and volume frequency (% of the fat) per μm class width against globule diameter. After P. Walstra, *Neth. Milk Dairy J.* **23** (1969) 99.

of dispersed fat to its surface area, and this is an important parameter in calculating the quantity of material that is required to cover the total fat surface area. Relative width is defined as c_s, the standard deviation in particle size divided by d_{vs}. Mean free distance x is the average linear distance a particle can move before touching another. Obviously, x depends closely on the fat content (see Fig. 3.2).

Volume surface average diameter d_{vs} is on average about 3.4 μm for milk

TABLE 3.1   Parameters of Frequency Size Distributions[1]

Parameter	Symbol	Given by	Dimension[3]
nth moment of distribution	S_n	$\sum N_i \cdot d_i^n$	$[L^{n-3}]$
Number average d	$\bar{d}$	S_1/S_0	$[L]$
Volume/surface average d	d_{vs}	S_3/S_2	$[L]$
Creaming parameter	H	S_5/S_3	$[L^2]$
Relative distribution width	c_s	$(S_2 S_4/S_3^2 - 1)^{1/2}$	—
Volume fraction of particles[2]	ϕ	$\pi S_3/6$	—
Specific surface area of particles	A	$\pi S_2 = 6\phi/d_{vs}$	$[L^{-1}]$
Mean free distance	x	$0.225 d_{vs}(0.74/\phi - 1)$	$[L]$

[1] $N_i/\Delta d$ is the number frequency; d is particle diameter.
[2] In milk, the fat content in % (w/w) is 100 $\phi \rho_{fat}/\rho_{milk} \approx 90\phi$ at 20°C.
[3] L means length.
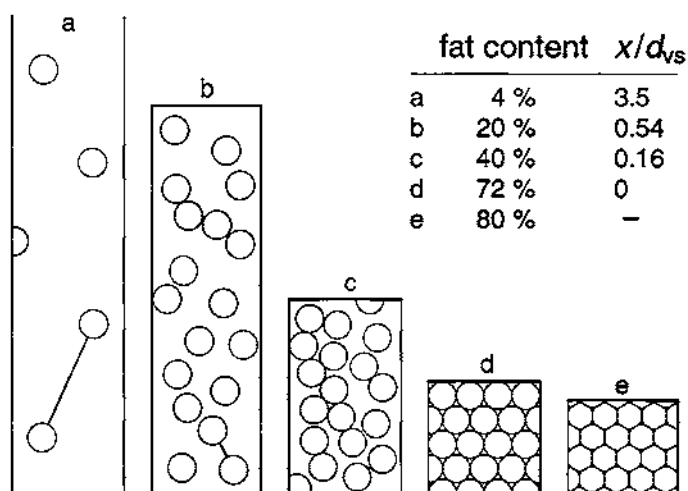
FIGURE 3.2 Distance between fat globules in milk and cream. Remember that a true representation cannot be given in a two-dimensional diagram. The line segments indicate the mean free distance *x*. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

of several breeds of cow, and c_s about 0.4. On average, 1 g of fat is divided among about 4×10^{11} globules, of which 10^{11} globules are >1 μm in diameter. Diameter d_{vs} varies among breeds (Jerseys have d_{vs} 4.5 μm), with stage of lactation (d_{vs} decreases on average from about 4.3 to 2.9 μm), etc. But the shape of the size distribution is almost constant. This implies that different size distributions can be made to coincide merely by altering the scales. Of course, the size distribution can be altered by treatment, especially by homogenization (Chapter 8). Quantitative data are given in Table 3.2.

3.1.1.2 Surface Layers

Each fat globule of milk is surrounded by a surface layer or membrane. The layer functions to prevent the fat globules from coalescence. Its composition is completely different from either milk fat or milk plasma, and is like that of a cell membrane, from which the fat globule membrane largely derives.

Table 3.3 gives the approximate composition of natural milk fat globule membrane, at least the main components. The phospholipids and cerebrosides have compositions similar to those given in Tables 2.9 and 2.10; they have many unsaturated fatty acid residues. The composition of the proteins of the membrane is intricate; there are at least 10 major species and several minor compounds. They are predominantly glycoproteins, specific for membranes, and include butyrophilin, which appears to

TABLE 3.2   Size Distribution of Fat Globules in Milk and Milk Products[1]

Product	Fat content (% w/w)	d_{vs} (µm)	c_s	A (cm²/ml product)	Mean free distance x (µm)	x/d_{vs}
Milk, Friesians	3.8	3.4	0.45	750	12.5	3.7
Milk, Jerseys	5.2	4.5	0.45	770	12.0	2.7
Homogenized milk	3.2	0.6	0.85	3500	2.5	4.5
UHT milk	3.2	0.3	0.85	7200	1.3	4.5
"Half-and-half"	10	3.5	0.45	1900	4.5	1.3
Light cream	20	1	0.95	13000	0.5	0.54
Heavy cream	40	4	0.5	6700	0.6	0.16
Evaporated milk	8	0.4	0.85	13000	0.6	1.6
Sweetened condensed milk	8	1	0.9	5300	1.1	1.1
Ice cream mix	12	0.6	0.9	13000	0.6	1.0
Separated milk	0.03[2]	1.2	0.5	17	600	500

[1] Approximate examples.
[2] Globular fat only.

be specific for milk fat globules. The membrane contains several other substances in trace quantities. Several of the membrane proteins are enzymes. Alkaline phosphatase and xanthine oxidase are of special importance; these enzymes make up a substantial part of the membrane proteins. The membrane is often said to contain many high-melting triglycerides, but that concept is poorly based. The presence of monoglycerides and free fatty acids in the membrane has been rather firmly established.

TABLE 3.3   Estimated Average Composition of the Membranes of Milk Fat Globules[1]

Component	mg per 100 g fat globules	mg per m² fat surface	Percentage of membrane material
Protein	1800	9.0	70
Phospholipids	650	3.2	25
Cerebrosides	80	0.4	3
Cholesterol	40	0.2	2
Neutral glycerides	+	+	?
Water	+	+	?
Carotenoids + vitamin A	0.04	2×10^{-4}	—
Fe	0.3	1.5×10^{-3}	—
Cu	0.01	5×10^{-5}	—
Total	>2570	>12.8	100

[1] Incomplete.

Hypothetical structures of the membrane have been given in several publications, but such pictures are very uncertain because membrane structure is poorly understood. The original structure of the membrane is presumably a lipid monolayer adsorbed from the cytoplasm surrounded by a layer of proteins and, on top of this, a lipid bilayer interspersed with proteins, some of which protrude into the milk plasma. However, much of this structure is lost during and after milk secretion and the membrane shows considerable variation from place to place. The average layer thickness is some 15 nm but varies from about 10 to 20 nm. The globules have a negative charge: ζ potential in freshly drawn milk is about -12 mV. The interfacial tension is $1-1.5$ mN $\cdot$ m^{-1}.

Material that is typical of the fat globule membrane is also found in skim milk, where it often amounts to about one-third of the total amount of membrane material present in milk (see also Tables 1.3 and 2.9). Not all of this originates from the fat globules but a part does, because treatment of milk causes the fat globules to lose membrane material. Coalescence of fat globules (Section 3.1.2) causes a decrease of their surface area, which leads to release of membrane material. Fat globules also lose part of their membrane if they come into contact with air (Section 3.1.3). Cooling leads to a migration of membrane material to milk plasma (the change is irreversible); about 20% of the phospholipids as well as some protein, xanthine oxidase, and Cu are released. By contrast, cooling causes adsorption of other proteins (cryoglobulins) onto the fat globules; but this process is reversible (Section 3.1.4).

Releasing part of the membrane from the surface area of a fat globule causes surface-active substances (mainly protein) to adsorb from the plasma onto the denuded fat−water interface. This may happen when air is beaten in (Section 3.1.3). Alternatively, increasing the fat surface area by reducing average globule size creates an uncovered interface, which subsequently acquires a coat of plasma proteins. This especially happens during homogenization (Chapter 8).

3.1.1.3 Crystallization

Crystallization of fat in fat globules differs from that of fat in bulk (Section 2.3). Supercooling must be deeper to induce crystallization. The crystals in a fat globule cannot grow larger than the globule diameter. The arrangement of the crystals may also be different from that of fat in bulk. If there are sufficient crystals in a globule, they can flocculate into a network that provides a certain rigidity to the globule. Sometimes, especially after churning, crystals tend to be sited in the fat−water interface and orient tangentially: this causes a bright layer in polarized light. As crystallization proceeds (e.g., by cooling), the tangentially oriented crystals may grow into a solid layer.

Crystallization of fat in the globules is of great importance in their coalescence stability (Section 3.1.2).

3.1.1.4 Differences Among Fat Globules

Differences among fat globules refer especially to their size (see above). Differences in size are associated with variations in composition. For example, the phospholipid content of fat globules will be almost inversely proportional to d_{vs}. However, globules of the same size also show variations in composition, especially with respect to triglycerides. There are considerable variations in composition among globules in one milking of one cow; for example, the final melting point of the globules in such milk can vary by up to 10 K. Membrane composition of individual fat globules can also vary, but quantitative data are not available.

3.1.2 Emulsion Stability

In many cases a stable emulsion is desired; the emulsion should not change on standing or during treatment. But moderate instability is desired during some treatments (e.g., in the whipping of cream and the freezing of ice cream); sometimes the emulsion should break, as in churning.

3.1.2.1 Types of Instability

Various types of physical instability can occur, as illustrated in Figure 3.3. The figure, of course, is schematic and simplified; the various changes may occur simultaneously. Some changes (e.g., creaming) always occur, though possibly slowly. In principle, flocculation and coalescence can occur spontaneously, but often the activation energy is high, so that these processes can be retarded or virtually prevented. Calculations based on the theory of the flocculation kinetics (see, e.g., Section 3.2.3.6) show that unhindered flocculation of fat globules followed by their coalescence would lead to separation of the fat in milk within a few minutes.

Fat globules can aggregate in various ways. Three types of aggregates are illustrated in Figure 3.4. We have given these types arbitrary names (others may use different names):

a. In floccules, attractive forces between globules are weak, and stirring disrupts the floccules. Flocculation does not happen normally with milk fat globules because electrostatic and steric repulsion prevents it. Milk fat globules do not flocculate even at their isoelectric pH (the isoelectric pH of ''washed'' milk fat globules is approximately 3.7). Some of the glycoproteins in the membrane cause sufficient steric repulsion. Agglutination, which refers to spontaneous flocculation in the cold, in raw milk is discussed in Section 3.1.4.2.

b. In clusters, two globules share part of the membrane material, generally micellar casein. Examples are so-called homogenization clusters (Sec-
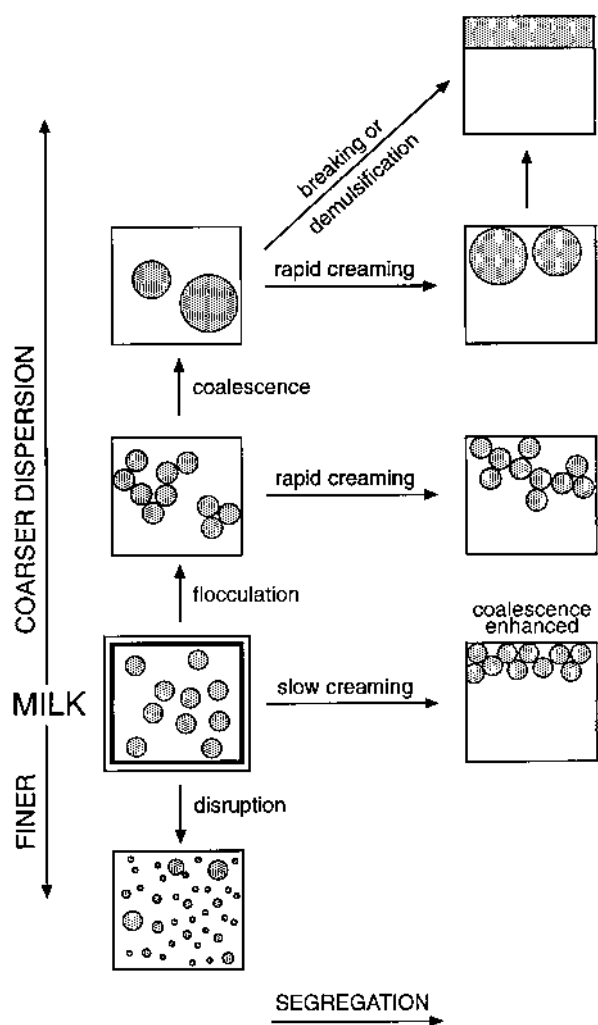
FIGURE 3.3 Types of physical changes in oil-in-water emulsions. Highly schematic. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

tion 8.7) and heat-coagulated fat globules (Section 6.2.4). Clusters usually cannot be disrupted by stirring.

   c.   In granules, fat touches fat. Aggregation to granules can only occur if the fat globules contain a network of fat crystals, giving the globules a certain rigidity. Granules usually cannot be disrupted.
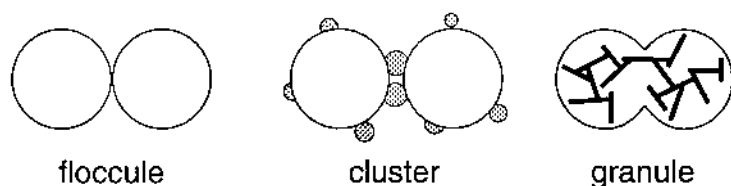
FIGURE 3.4 Different types of aggregates of fat globules. Gray dots denote (parts of) casein micelles; heavy lines denote fat crystals. Highly schematic, not to scale.

3.1.2.2 Partial Coalescence

When two emulsion droplets are close together and the film between them has been decreased to a few nm, the film can be ruptured. Consequently, the globules can fuse or coalesce into one droplet. But the globules usually have a surface layer that causes sufficient repulsion between them to prohibit close approach, hence coalescence. The globules may contain fat crystals of which some may stick somewhat out of the globule. If so, such a protruding crystal may pierce the film between two approaching globules, usually leading to a commencing coalescence. A complete coalescence cannot occur because the crystals in the globules prevent this, and a granule is formed. The process therefore is called partial coalescence. True coalescence is rarely an important process in milk products. This is because of the high stability of milk fat globules, though coalescence may occur in unhomogenized cream at sterilization temperature. Partial coalescence, however, can easily occur, at least if part of the fat is solid, and especially during vigorous flow of the product.

Partial coalescence (formation of granules, clumping) differs definitely from true coalescence in its effects. In the latter case, small fat globules transform into larger ones, in the former into large, irregularly shaped granules (''butter grains'') or even a continuous network as in whipped cream. We also may envision a granule of two fat globules as a collection of separate, rigid globules held together by a ''neck'' of liquid fat between them. Often crystals will be present in these necks, and sometimes the original fat globules can no longer be distinguished.

Increasing the temperature and thereby melting the fat crystals will cause coalescence of the formed granules into (large) droplets. Consequently, the oil–water interface decreases and substances of the fat globule membrane (e.g., phospholipids) are released into the plasma. During partial coalescence release of membrane components may also occur, although to a lesser degree.

Often the terms ''free fat,'' or even ''uncovered fat,'' are used to describe occurrence of visible or invisible clumping or coalescence. Uncovered fat as such

cannot exist in milk because there is a large excess of surface-active material present in milk plasma. Fat globules or granules will immediately (i.e., within 0.1 s) acquire this material at their damaged spots. Incipient granule formation can only be detected by examining whether the average particle diameter has increased.

The following are factors affecting the rate of partial coalescence.

a. Stirring or, more precisely, bringing about a velocity gradient or shear rate in the liquid has a considerable effect (Fig. 3.5). This is typical of partial coalescence because stirring usually has little or no effect on coalescence if the globules are liquid. The velocity gradient increases the rate of encounters of the fat globules, causes encountering globules to roll around each other (enabling a protruding crystal to pierce the film between the globules), and can bring them closer together (enabling crystals that protrude less far to pierce the film).

b. Beating in of air is again a way of stirring; moreover, it enhances clumping in another way (see Section 3.1.3).

c. Fat content has considerable effect (Fig. 3.5). This is because partial coalescence follows second-order kinetics.

d. The proportion of solid fat is crucial (see Fig. 3.6). If there are no crystals, partial coalescence cannot occur. If the globules contain too much solid fat (e.g., after keeping them for some hours at $<5°C$), the residual liquid fat is retained in the pores of the crystal network, leaving no sticking agent to hold the globules together.
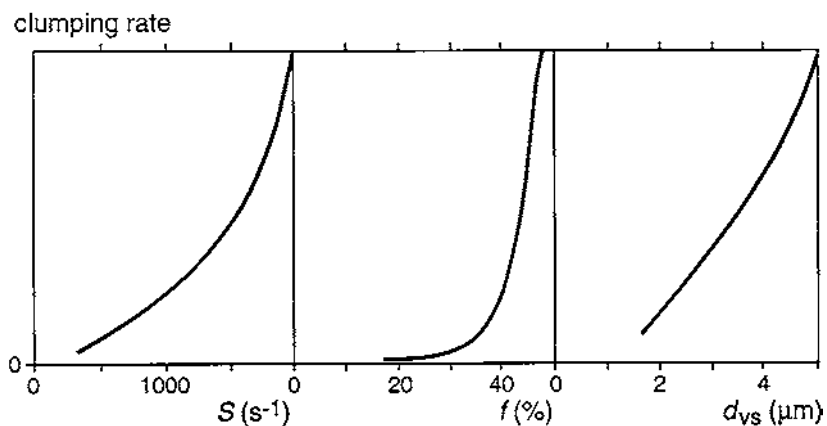
FIGURE 3.5 Rate of clumping (or of churning) of cream as a function of shear rate (*S*), fat content (*f*), and average fat globule size (d_{vs}). Approximate results.
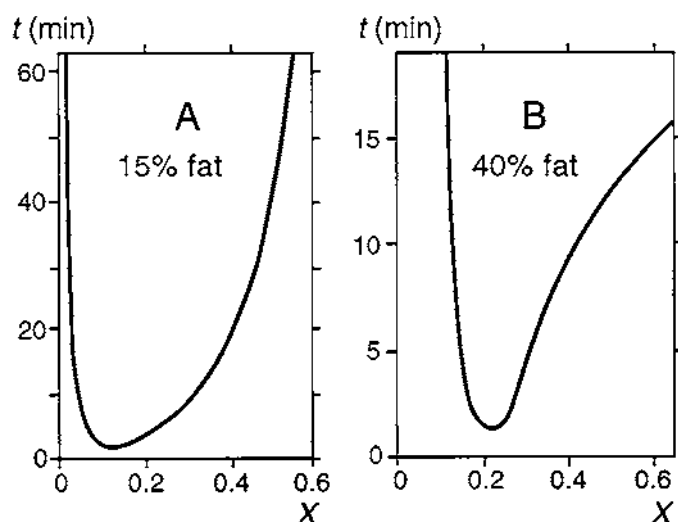
FIGURE 3.6 Effect of the average proportion of solid fat (x) in the fat globules on the "churning time" (t = time needed to form visible granules). (A) Churning with air. (B) Stirring in the absence of air (velocity gradient $S \approx 1500$ s^{-1}). Approximate results.

e. The smaller the globules, the stabler they are (see Fig. 3.5). Larger globules have larger crystals, and therefore the probability of a crystal sticking far enough out of the globules to pierce the film between two globules is increased. For larger globules, fewer aggregation events are needed to form visible granules. The effect of globule size is considerable. For example, homogenized cream cannot be churned. Usually, the globule size is, ceteris paribus, the main cause of variation in coalescence stability if different emulsions are compared.

f. The surface layers of the fat globules are also essential. Natural fat globules are reasonably stable. But if the globules of a similar size have a surface layer of protein, as occurs after homogenization or recombination (Section 8.5), they are far more stable. Displacing part of that proteinaceous surface layer by adding surfactants like monoglycerides or Tweens markedly decreases the stability to coalescence. Such surfactants therefore are commonly included in ice cream, in which the globules should aggregate during the beating process.

g. Temperature fluctuations can have a considerable effect. For instance, keeping cream of 25% fat or more at 5°C for some time, then warming to about 30°C (e.g., 30 min) and cooling it again—not very rapidly—

produces a strong increase in its viscosity, and it may even gel. This process is called rebodying. For this to happen, warming to a temperature at which not all but most of the crystals melt is paramount. Rebodying is caused by partial coalescence. It only occurs without stirring if the fat content is so high that the fat globules are very close together. It may also occur in a cream layer formed on high-pasteurized unhomogenized milk.

Size, orientation, and network formation of the crystals in the globules will have a significant effect on the tendency to form granules. An example is the above-mentioned rebodying.

3.1.2.3 Disruption

Disruption, i.e., breaking up of the fat globules into smaller ones, does not happen spontaneously. Beating in of air, intense turbulence as applied in a high-pressure homogenizer, or a very high velocity gradient (S, in s^{-1}) all may cause disruption. To achieve this, the shearing stress (ηS, where viscosity of plasma $\eta \sim 1$ mPa · s) exerted on a globule of diameter d should exceed the resistance to deformation as caused by the Laplace pressure ($4\gamma/d$, where surface tension γ between globule and plasma is about 1.5 mN · m^{-1} for natural milk fat globules). In other words, the smaller the globules, the more difficult the disruption. Therefore, to disrupt natural fat globules, S should be excessively high, i.e., on the order of 10^6 s^{-1}. Even vigorous stirring of milk will thus not disrupt fat globules, but intense beating in of air will do so.

3.1.3 Interactions with Air Bubbles

Skim milk foams readily, especially at low temperature. The foam is most stable at a somewhat higher temperature, say, 40°C. Proteins, above all casein, stabilize the foam lamellae. Milk fat depresses foaming. For example, addition of 1% whole milk diminishes the foaming tendency of separated milk to less than half. Apparently, the milk fat globules affect the foam lamellae.

Figure 3.7 shows what may happen when an emulsion droplet approaches an air–water interface. Phenomena depend on the value of the spreading pressure and on the presence of layers adsorbed onto the different interfaces, as illustrated. When a milk fat globule makes contact with a newly formed air–water interface, membrane material of the droplet and thereafter part of its contents will spread over the interface. This happens as long as no other surface-active substances, especially proteins, have been adsorbed onto the interface. The spreading rate is about 0.1–1 mm/s, whereas adsorption of surface-active substances from plasma onto air–water interfaces takes about 0.01–0.1 s. Hence, a spreading of the order of 10 µm radius would be possible. Clearly, globules can become attached to the

A

$$P_S = \gamma_{WA} - (\gamma_{OW} + \gamma_{OA}) < 0$$

$$P_S > 0$$

$$P_S > 0, \text{ interfacial layers}$$
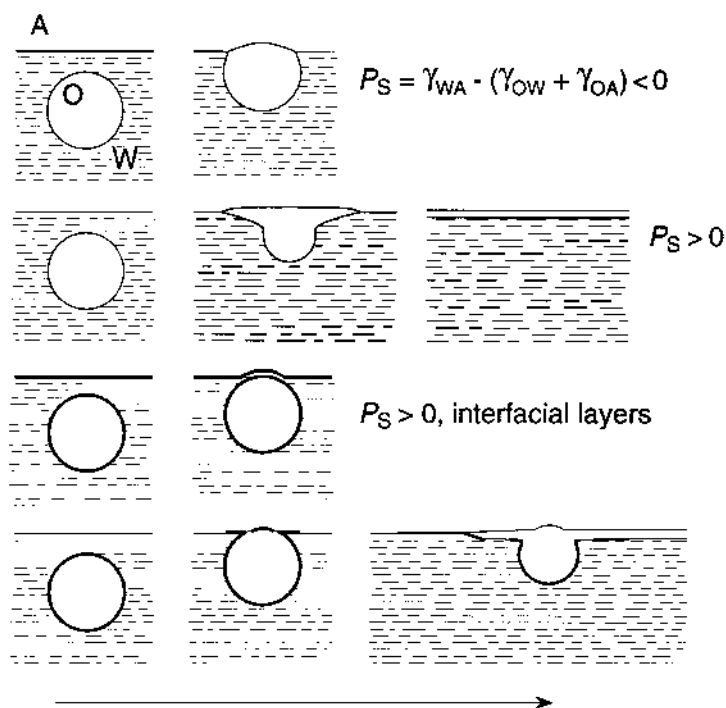
FIGURE 3.7 Interactions between an oil droplet (O) and the air–water (AW) interface, as a function of the spreading pressure (P_s; γ = interfacial tension) and of the presence of interfacial adsorption layers (heavy lines). The bottom row refers to a milk fat globule contacting a newly formed air–plasma interface. Schematic, not to scale. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

air–water interface, and thus to an air bubble. In this way, collection of globules in a foam can occur; this process may be called flotation.

A fat globule can make contact with an air bubble having an adsorption layer or it can be caught between two such bubbles, i.e., in a foam lamella. In these cases, electrostatic and steric repulsion between globule and air–water surface layer usually prevents the globule from making contact with the air in the bubble; in other words, the globule cannot pierce the surface layer. However, globules containing fat crystals may pierce this layer and make contact with air (see also Section 3.1.2.2), causing membrane material and fat to spread over the interface. The spreading usually causes rupture of the foam lamella. Small fat

globules as well as fully liquid globules show less tendency to make a foam unstable because these pierce the adsorption layer of the air bubble less readily.

Rapid beating in of air in milk or cream (as in churning or whipping) causes new air–water interface to be continually formed, and fat may spread over the interface. If the fat is fully liquid, the subsequent breaking up of air bubbles covered with fat causes disruption of the fat. Churning warm milk or cream thus yields smaller fat globules. If the globules also contain solid fat, they become attached to the air bubbles. As the air surface area diminishes (because air bubbles coalesce), the attached fat globules are driven nearer to each other; the liquid fat spread over the air bubble surface readily causes the globules to form granules (see also Fig. 3.6). Furthermore, the liquid fat makes a foam less stable; in other words, the lifetime of the air bubbles is short. Further aggregation of granules yields butter grains, in which a phase inversion has apparently taken place, i.e., oil is the continuous phase. But the grains still contain fat globules and moisture droplets. Concentrating and then working the grains removes excessive moisture and reduces the moisture droplets in size. In this way, butter is obtained (Chapter 19).

If there is very little liquid fat during beating in of air and if, moreover, the fat content is fairly high, structures of fat clumps are formed. However, churning does not occur; the structures entrap the air bubbles, so that whipped cream is obtained. Similar processes occur during freezing and whipping of ice cream.

3.1.4 Creaming

Because of the difference in density between milk plasma and fat globules, the globules tend to rise. This property is of great importance because it causes (undesirable) creaming during keeping and enables milk to be separated into cream and skim milk. Creaming is much enhanced if the fat globules have been aggregated into flocculas or clusters.

3.1.4.1 High-Pasteurized Milk

In this milk, creaming of single fat globules may occur. The velocity v_s of a rising globule is usually obtained from Stokes's equation:

$$v_s = -a(\rho_p - \rho_f)d^2/18\eta_p \tag{3.1}$$

where ρ_p is density of plasma, ρ_f is density of fat globules, η_p is viscosity of plasma (not of the milk); a is acceleration, i.e., g if creaming is due to gravity. For Stokes's law to hold several conditions must be fulfilled, but the equation is quite useful to predict trends.

In practice, globule size and temperature mainly determine the extent of creaming in high-pasteurized milk. In first approximation, the creaming rate is proportional to the creaming parameter H (Table 3.1). H varies among lots of

TABLE 3.4   Density (ρ) and Viscosity (η) of Milk Fractions as a Function of Temperature (T)[1]

T (°C)	ρ_{plasma} (g · ml^{-1})	ρ_{fat}[2] (g · ml^{-1})	η_{plasma} (mPa · s)	η_{water} (mPa · s)
5	1.0359	0.959	2.83	1.519
10	1.0352	0.951	2.35	1.307
15	1.0344	0.938	1.99	1.139
20	1.0333	0.916	1.68	1.002
30	1.0300	0.909	1.26	0.798
40	1.0261	0.902	1.00	0.653
60	1.0166	0.889	0.69	0.466

[1] Approximate averages.
[2] Values for $T < 40$°C are calculated by taking some supercooling of the fat into account.

milk but processing of milk, especially homogenization, is the main variable. Data with respect to H are given in Tables 8.1 and 8.3. Temperature affects factor $(\rho_p - \rho_f)/\eta_p$ (Table 3.4; Fig. 7.2B).

Clusters of fat globules cream much faster than single globules. Clustering may be caused by homogenization (Section 8.7) or by intense heating (sterilization). The latter clustering can occur in evaporated milk where it causes undesirable creaming.

3.1.4.2   Raw Milk

The creaming in the cold is usually determined by the flocculation of the globules by ''agglutinin,'' i.e., a complex of cryoglobulins (predominantly immunoglobulin M) and lipoproteins; see also Section 2.4.3. In cold agglutination the following events may be envisioned (Fig. 3.8), though the actual mechanism of creaming probably is more complicated.
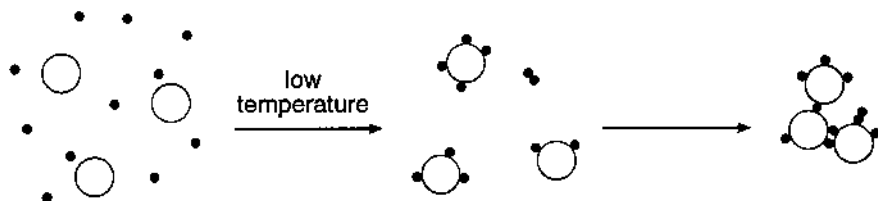
FIGURE 3.8   Adsorption of ''agglutinin'' (black dots) onto milk fat globules and the ensuing flocculation of the globules. Highly schematic.

a. In the cold, cryoglobulins precipitate onto all kinds of particles, especially fat globules (at a rate about 10^{-3} times that predicted by the theory for fast flocculation).
b. The so ''covered'' fat globules aggregate into fairly large floccules.
c. Large floccules rise rapidly.
d. Large floccules overtake smaller ones and single fat globules, thereby enhancing flocculation and rising still faster. In this way, a cream layer forms rapidly, even in a deep vessel or a large milk tank.

Following are the main variables affecting natural creaming in raw milk (Fig. 3.9).

a. *Temperature*. No creaming occurs at 37°C. The colder the milk, the quicker the creaming is.
b. *Concentration of agglutinin* varies among cows and with lactation stage, i.e., high in colostrum, negligible in late lactation milk.
c. *Fat globule size*, partly because smaller globules have a relatively larger surface area and, consequently, need more agglutinin.
d. *Fat content*. The higher the fat content of milk, the quicker the creaming is because formation of floccules is quicker.
e. *Agitating* milk for a while at low temperature seriously impairs creaming. A possible explanation is that aggregates of agglutinin are formed causing a decrease of the number of active particles.
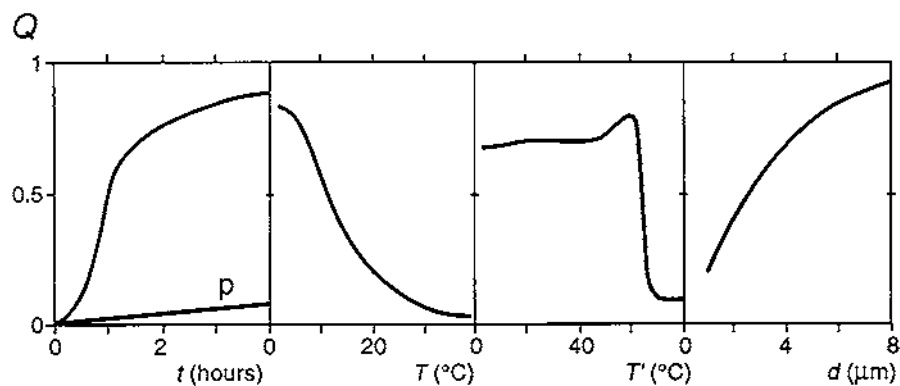
FIGURE 3.9 Gravity creaming in raw milk. Effect of creaming time (*t*), creaming temperature (*T*), temperature of pretreatment (*T′*, during 30 min), and globule size (*d*) on fat fraction creamed (*Q*). p = high-pasteurized milk. Approximate examples. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

    f.   *Warming* such milk largely restores the ability to flocculate because it dissociates agglutinin from the aggregates.

    g.   *Heat treatment* of milk can inactivate agglutinin. The effect precisely parallels the insolubilization (by denaturation) of the immunoglobulins. Heating for 20 s at 71°C has no effect, 73°C causes 25% less creaming, and 78°C often leads to complete inactivation (Fig. 2.25).

    h.   *Homogenization* inactivates agglutinin, also if it takes place without the fat globules being present. The explanation for this is unknown.

3.1.4.3 Cream and Skim Milk

Cold agglutination in raw or low-pasteurized milk leads to a deep cream layer of loosely packed floccules, containing much plasma; the layer may contain 20% to 25% fat. The cream can readily be redispersed throughout the milk. It contains many of the bacteria from the milk. In low-pasteurized cream of 20% fat or higher, creaming will hardly occur: the globules aggregate into one large floccule that fills the whole volume. However, gravity tends to compact this flocculated system and a thin layer of plasma may form at the bottom, slowly increasing in height.

    A different cream layer forms on high-heated milk. This is a thin layer of a high fat content (40% to 50%), but the closest possible packing of globules (about 70% fat) is not attained. When the fat starts crystallizing in the cream layer, this may promote partial coalescence of the fat globules, and so does stirring the layer. Consequently, it may then be impossible to redisperse the cream throughout the milk. By centrifugal action, cream with a much higher fat content, i.e., 80% fat or more (''plastic'' cream), can be obtained so that globules are deformed (Fig. 3.2).

    Skim milk obtained after natural creaming rarely has a fat content of less than 0.5% (though a content as low as 0.1% may be achieved under ideal conditions); the milk still contains fairly large globules. Centrifugally separated milk has a far lower fat content, made up by the smallest fat globules and about 0.025% ''nonglobular fat.''

    Most of the agglutinin is found in the cream if raw or low-pasteurized milk is separated when cold, e.g., 5°C. This can cause considerable agglutination in the cream. Most of the agglutinin is in the skim milk if the milk is separated when warm, say, >35°C. Consequently, a mixture of cold-separated skim milk and of cream obtained by warm separation displays hardly any cold agglutination.

3.1.5 Lipolysis

In milk, lipolysis (i.e., enzymatic hydrolysis of triacyl glycerides) causes free fatty acids to be formed, and this may give the milk a soapy-rancid taste. Several

enzymes can be responsible for lipolysis, but here we consider the main lipolytic enzyme of milk, i.e., lipoprotein lipase (Section 2.5.2.4). In blood, this enzyme liberates fatty acids from lipoproteins and chylomicrons. A cofactor, in the present case an apoprotein from certain lipoproteins, is necessary for the enzyme to act at the oil–water interface. Optimum temperature of the enzyme is about 33°C, optimum pH about 8.5. Milk contains 10–20 nmol of the enzyme per liter. Under optimal conditions, k_{cat} is greater than 3000 s^{-1} and would suffice to make milk rancid in 10 s, but this never happens.

There are several factors that slow down or enhance lipolysis in milk. The situation with respect to lipolysis is highly intricate and may be as follows:

a. Ionic strength, ionic composition, and pH of milk are suboptimal.

b. The enzyme is bound largely to the casein micelles. This diminishes its activity considerably. Factors a and b would cause k_{cat} in milk to be reduced to some 100 s^{-1}, which can induce perceptible rancidity in milk in 5 min. In exceptional cases, this indeed may happen (see below).

c. The natural fat globule membrane protects the inside of fat globules against enzyme attack. The cause is the very low interfacial tension between oil and water, usually <2 mN · m^{-1}. Because the enzyme alone cannot produce such a low interfacial tension, it cannot penetrate the membrane to adsorb onto the fat. (A component can only adsorb onto an interface if it reduces the interfacial tension.)

d. Together with certain lipoproteins from blood (the apoproteins alone probably do not suffice), the lipase can adsorb onto the fat. Addition of blood serum to milk causes fast lipolysis.

e. Milk contains one or more enzyme inhibitors (besides the casein micelles) that presumably counteract the stimulating lipoproteins. Probably, proteose-peptone component 3 is the most important inhibitor.

f. Product inhibition occurs, although not because of the free fatty acids formed. Probably long-chain saturated monoglycerides, if formed in fairly high concentration, act as inhibitor. Because these molecules cannot diffuse, or very sluggishly diffuse, away from one fat globule to the other, product inhibition may be a local event.

g. Temperature affects the partitioning of enzyme activity (enzyme, stimulator and/or inhibitor) among the fractions. At low temperature more enzyme activity is associated with the fat globules. This may explain why in raw milk the optimum temperature for lipolysis generally is about 15°C.

h. The enzyme is slowly inactivated in raw milk.

Most raw milk scarcely becomes rancid on standing, though the milk of some cows does. Increased fat acidity especially occurs when milk yield

becomes low (<3 kg/milking), i.e., at the very end of lactation. As is illustrated in Figure 1.9C, this causes seasonal variation in degree of lipolysis. Increasing the number of milkings also leads to increased lipolysis. All of these conditions enhance ''leakage'' of lipoproteins from the blood into the milk. Some milks of individual cows are highly susceptible to lipolysis, leading to ''spontaneous lipolysis.'' But addition of ''normal'' to ''susceptible'' milk considerably slows down lipolysis, as is shown in Figure 3.10. This is because inhibitors are present; consequently mixed milk, if treated properly, rarely becomes rancid.

Lipolysis can, however, readily be induced in milk. Removing the natural membrane from the fat globules by intense beating in of air (Section 3.1.3), or increasing the fat–plasma interface by homogenization, leads to a globule surface layer of plasma proteins (Section 8.5), which causes an interfacial tension up to $15 \, \text{mN} \cdot \text{m}^{-1}$. As a result, lipoprotein lipase can penetrate the membrane. Homogenization of raw milk thus causes very rapid lipolysis.

Lipolysis can also be induced by cooling raw milk to 5°C, warming it to 30°C, and cooling it again, although there is wide variation among milk samples. The explanation is uncertain.

Inactivation of the enzyme by heating (Figs. 2.25, 14.2, 22.1) can prevent lipolysis.
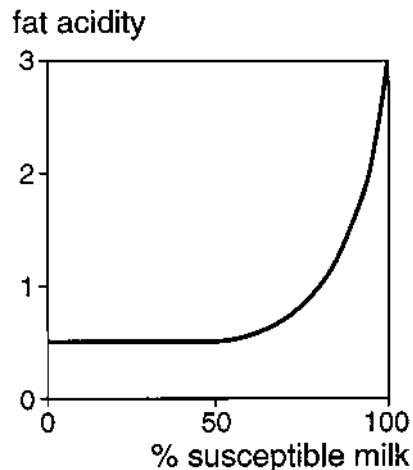
FIGURE 3.10 Acidity of milk fat (as mmol/100 g) of milk ''susceptible'' to lipolysis, ''normal'' milk, and mixtures thereof. The milks were kept for 24 h at 4°C. After unpublished results of A. Jellema.

3.2 CASEIN MICELLES

The fact that casein in milk is not present in solution but in micelles has important consequences for the properties of milk. To a large extent the casein micelles determine the physical stability of milk products during heat treatment, concentrating, and holding. Their behavior is essential in the first stages of cheese making. The micelles largely determine the rheological properties of sour and concentrated milk products. The interaction of casein micelles with oil–water interfaces is of importance with respect to properties of homogenized milk products.

3.2.1 Description

Almost all casein in fresh uncooled milk is present in roughly spherical particles, mostly 40–300 nm in diameter. On average, the particles comprise approximately 10^4 casein molecules. These casein micelles also contain inorganic matter, mainly calcium phosphate, about 8 g/100 g casein (Table 2.3). They contain also small quantities of some other proteins, such as part of the proteose-peptone and certain enzymes. The micelles are voluminous, holding more water than dry matter. They have a negative charge.

3.2.1.1 Submicelles

When casein is in a solution comparable with milk serum, but at low calcium ion activity, small and roughly spherical aggregates form. These are about 12–15 nm in diameter, and each of them contains 20–25 casein molecules. In these so-called submicelles, hydrophobic bonds and salt bridges (plus–minus interactions) keep the molecules together. Presumably, most hydrophobic parts of the molecules are buried in the core of the submicelles, whereas many charged groups would be in a less hydrophobic outer layer.

Each submicelle contains different casein molecules, but not all submicelles have the same composition. Essentially, there are two major types of submicelles, with and without (or with little) κ-casein. This is not surprising, considering that (a) the submicelles contain at the most 25 protein molecules; (b) the molar ratio being $\alpha_{s1}:\alpha_{s2}:(\beta + \gamma):\kappa \approx 4:1:4:1.6$; and (c) κ-casein exists in milk as a polymer, on average consisting of six molecules held together by —S—S— linkages. Presumably, the C-terminal hydrophilic part of κ-casein, which may contain sugar residues, is sticking out from the submicellar surface.

Adding an excess of calcium and phosphate, as occurs in the mammary secretory cells, results in aggregation of the submicelles into larger units, i.e., the casein micelles. The explanation of this aggregation presumably is that deposition of calcium phosphate in the submicelles lowers their electric charge and also makes them more compact. Consequently, the submicelles would attract each

other, albeit weakly, causing their aggregation. Two submicelles that approach each other in such a way that the protruding ''hairs'' of κ-casein of at least one of them are in between will not be able to become bound to each other. This would imply that the aggregation of submicelles goes on until a roughly spherical aggregate is formed, whereas the surface of this micelle is covered with a more or less continuous layer of hairs of κ-casein. If other submicelles (or their aggregates) approach, they cannot become bound to the micelle formed. The hydrodynamic thickness of the hairy layer is about 7 nm.

One can mimic the above synthesis of casein micelles in vitro by dissolving casein in simulated milk serum and subsequently adding—slowly and at constant pH—Ca, Mg, phosphate, and citrate in the right proportions. As a result, the formed micelles have almost all of the properties of native micelles. The properties of the synthetic micelles can to a certain extent be varied by varying their composition.

3.2.1.2   Model

Figure 3.11 depicts a model of the casein micelle derived from the above reasoning. The micelle is a fairly dense aggregate of submicelles. The latter contain small regions (often called nanoclusters) of calcium phosphate in which the serine phosphate residues of the casein are involved. Considerable portions of the pep-
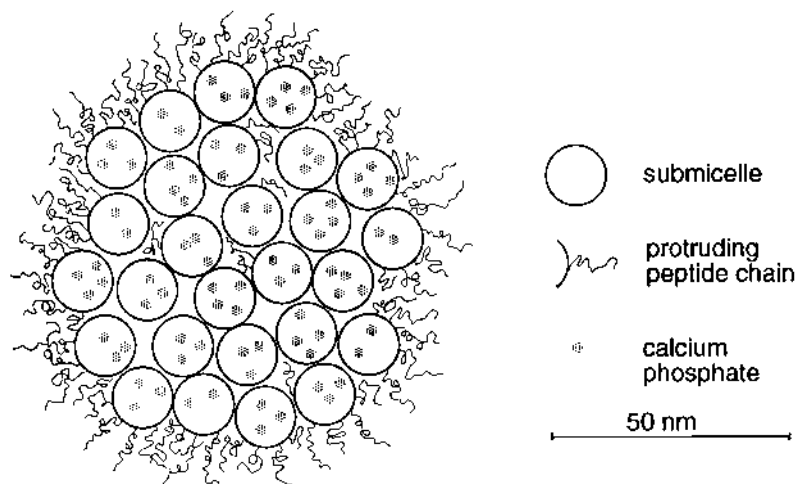
FIGURE 3.11   Schematic model of a cross-section through a casein micelle. Mainly after D.G. Schmidt and T.A.J. Payens, *Surface and Colloid Science* **9** (1976) 165; W. Slattery, *Biophys. Chem.* **6** (1977) 59; P. Walstra, *J. Dairy Res.* **46** (1979) 317 and *Int. Dairy J.* (in press).

tide chains of casein in loose submicelles have great freedom of motion. When calcium phosphate is present, as occurs in the whole micelle, the flexibility of the peptide chains is almost fully lost, except that of the above C-terminal part of κ-casein (from about residue 90). Most of these parts are sticking out from the micelle into the solution as flexible ''hairs,'' which are essential in providing stability to the micelles (see Section 3.2.3). Further information on the colloidal calcium phosphate (CCP), i.e., the calcium phosphate included in the micelles, is given in Section 2.2.3. The submicelles should not be conceived as rigid spherical particles, but they will be somewhat irregularly shaped and fuzzy. Possibly, protein–protein contacts also exist between the submicelles.

The micelles are fairly voluminous, but experimental results on their voluminosity vary widely. Presumably, the results involved may be interpreted such that the voluminosity of the micelles without the hairy layer is some 2–2.5 ml/g dry casein. Dry casein would occupy about 0.7 ml/g, so that the rest of that volume is water, partly in and partly between the submicelles. Taking the hairy outer layer into consideration, micelle voluminosity is about 4 ml/g of casein. Hence, the total volume fraction of casein micelles in milk approximates 0.1. Small solute molecules can penetrate the micelles, but large ones like serum proteins can do so hardly if at all. Obviously, the liquid in the micelles is not water only, but neither is it identical to milk serum.

3.2.1.3 Variability

The micelles are not all the same. They show a distinct size distribution, a typical example being given in Figure 3.12. Note the great number of very small particles. These are loose submicelles, which make up only a small part of the total casein. Authors do not fully agree about the size distribution. Some have found a small number of very large micelles, up to 600 nm diameter. We estimate the volume-surface average diameter (d_{vs}), excluding the loose submicelles, to be at least 100 nm. Further data are given in Table 3.5. Two kinds of variation should be distinguished: (a) the variation between the micelles of one milking of one cow and (b) the variation between different lots of milk, from different cows, etc. Different cows produce milk with a different size distribution. This is roughly represented in Table 3.6.

Table 3.6 shows that the protein composition is also variable. In particular, the proportion of κ-casein varies; it largely determines the average casein micelle size. Because κ-casein is at the surface of the micelles, its content must be expected to be inversely related to d_{vs}; this is confirmed by experiments. (It does not imply that there is only κ-casein at the surface of the micelles. Moreover, a small part of the κ-casein may be in the interior of the micelles.) The voluminosity of the micelles also varies. It will markedly increase with decreasing micelle size because of the constant thickness (approximately 7 nm) of the hairy layer and
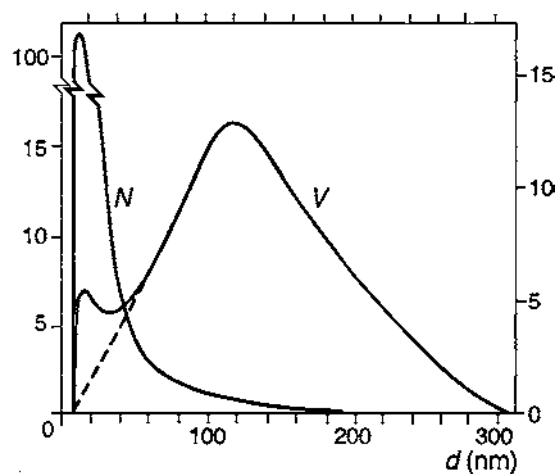
FIGURE 3.12 Example of the size frequency distribution of casein micelles. Number (*N*, left-hand ordinate) and volume frequency (*V*, right-hand ordinate), both in percentage of the total per 20-nm class width, against micelle diameter (*d*). After D.G. Schmidt, P. Walstra, and W. Buchheim, *Neth. Milk Dairy J.* **27** (1973) 128.

TABLE 3.5 Size Distribution of Casein Micelles[1]

Parameter	Full distribution	Without particles <20 nm
Number per μm^3 milk	600	120
Volume fraction, ϕ	0.06[2]	
Number average diameter, $\bar{d}$ (nm)	25	65
Volume-surface average diameter, d_{vs} (nm)	86	104
Width[3]	0.45	
Mean free distance, x (nm)	130	150
Surface area (m^2/ml milk)[4]	4.2	3.5
Same of submicelles (m^2/ml milk)	23	

[1] Approximate example of parameters of the distribution.
[2] Including the hairy layer: 0.1.
[3] Standard deviation/average of the volume distribution.
[4] Excluding the hairy layer and assuming a spherical shape.

TABLE 3.6 The Extent of Variation in the Properties of Casein Micelles[1]

Property	Variation	
	Within milk	Among milks
Particle size	$+++$	$+$
Voluminosity	$++$[2]	$++$[2]
Protein composition	$++$[2,3]	$+$[2,3]
Amount of colloidal phosphate	$+$	$+$
Composition of colloidal phosphate	?	$+$

[1] Approximate results of the variability between the micelles of one milking and between different milks, respectively.
[2] Is partly linked with micelle size.
[3] Mainly because of variable κ-casein content.

the low protein content in that layer. The content and composition of the colloidal calcium phosphate may vary, too. Large micelles probably have a higher CCP content.

3.2.2 Changes

During keeping of milk the casein micelles alter, though slowly. This is because there is no true state of thermodynamic equilibrium between the micelles and their surroundings. The main change probably is proteolysis of β-casein into γ-casein and proteose-peptone by plasmin (Section 2.5.2); part of the proteose-peptone enters the serum. Even from a purely physicochemical point of view, the micelles are not stable. The main cause is that the colloidal phosphate is not in the stablest form. The phosphate thus will (usually slowly) be converted to stabler phosphates (brushite, octa calcium phosphate, or hydroxyapatite, depending on conditions), associated with the casein in another way or in the form of a precipitate that is separate from the micelles.

Furthermore, the casein micelles will alter during changes in the external conditions, especially temperature and pH. Some of these alterations are reversible, whereas others are not or partly so.

3.2.2.1 Dynamic Equilibria

A casein micelle and its surroundings keep exchanging components. The principal exchanges are represented schematically in Figure 3.13. The exchanges can be considered dynamic equilibria, though they may be pseudo- rather than true equilibria.

The mineral compounds exchange the fastest. The counterions, present as free ions in the electrical double layer, would exchange very rapidly. Some of
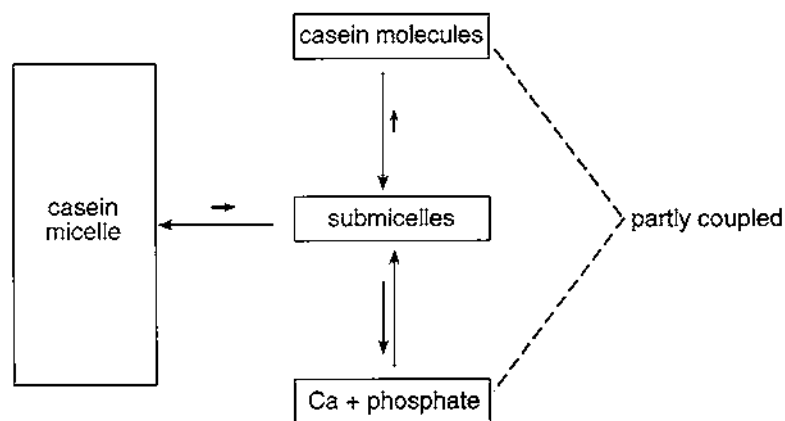
FIGURE 3.13 Outline of the main dynamic equilibria between casein micelles and serum.

the components of the colloidal phosphate, including Ca, Mg, phosphate, and citrate, also exchange fairly rapidly (relaxation time, e.g., 1 h), while the remaining part appears to be strongly bound. Submicelles can diffuse in and out of each micelle, and the (average) equilibrium situation depends on such factors as temperature, pH, and a_{Ca}^{2+}. The size distribution is resultant of a continuous process of aggregation and disintegration. Casein micelles can be broken up to smaller units by mechanical forces, e.g., by very intensive homogenization; the formed fragments then rapidly reaggregate (relaxation time a few minutes) into the original size distribution.

All of this means that a model of the casein micelle, as depicted in Figure 3.11, would represent an instantaneous picture, a ''snapshot'' as it were, with an exposure time of about 10^{-12} s. The protruding hairs would show the fastest motion. Small changes in micelle shape would take less than 1 s. At a time scale of 1 min, some submicelles would leave the micelle and new ones would be incorporated. Diffusion of a submicelle from one micelle to a neighboring one would take a time of the order of 1 ms.

Free casein molecules also occur in milk, though they comprise only a tiny part of the casein at physiological temperature. At low temperatures, their amount increases considerably (see below).

The relative rate of most of the exchanges strongly decreases with decreasing temperature. Although the processes may be reversible to the extent that compounds that have left the micelles can return to them (e.g., when increasing temperature again), it remains to be seen as to whether this is a return to the native micelle structure.
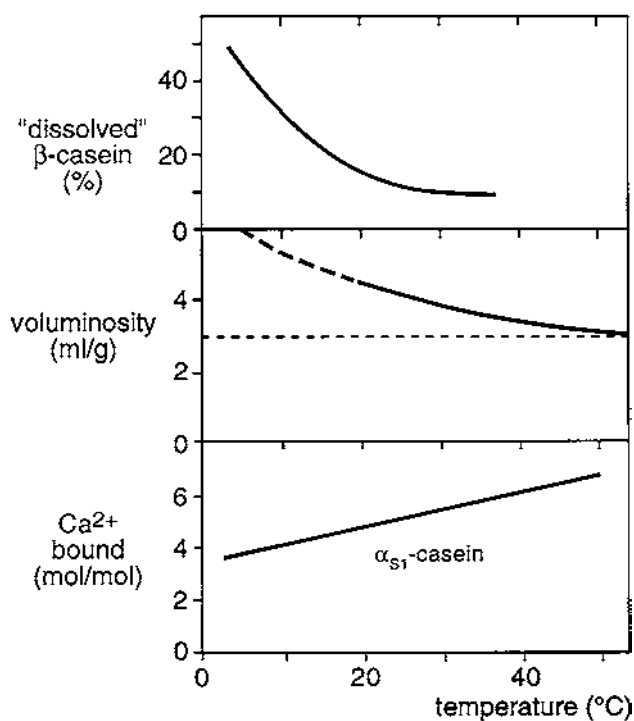
FIGURE 3.14 Influence of the temperature (after keeping milk for about 24 h) on some properties of casein micelles. Percentage of the β-casein not associated with the micelles. Voluminosity (in ml/g dry casein) from intrinsic viscosity; below 20°C (broken line) the values are probably overestimated. Binding of Ca^{2+} ions to α_{s1}-casein. Results at physiological pH.

Furthermore, the voluminosity of the micelles and their electrostatic charge may vary, usually as a result of the above-mentioned changes.

3.2.2.2 Low Temperature

Figure 3.14 illustrates some changes that occur by lowering the temperature. Dissolution of a considerable part of the β-casein occurs. But it is important to note that essentially a part of the dissolved β-casein is included in loose submicelles (experimentally differentiating between the two states of β-casein is difficult). Undoubtedly, the main cause of the dissolution of β-casein is that the hydrophobic bonds, which are predominantly responsible for its binding in the submicelles, are much weaker at low temperature. It therefore is not surprising

that other caseins will dissolve as well, although to a lesser extent (α_s-caseins least); see also Figure 3.15. Proteolytic enzymes can far better attack casein in a dissolved state. Because of this, for example, β-casein is fairly rapidly converted by plasmin at low temperature.

As is illustrated in Figure 3.14, the voluminosity of the micelles increases markedly at low temperature. This increase should be largely ascribed to the formation of another category of hairs. In addition to some β-casein molecules going into solution, others may also be loosened so that β-casein chains now may protrude from the core surface of the micelles. This hairy layer is discussed further in Section 3.2.3.2. The voluminosity of the core of the micelles may also increase. Finally, a limited disintegration of micelles into smaller ones appears to occur, which would also cause the average voluminosity to increase.

Disintegration of micelles upon cooling may also be due to dissolution of a part of the CCP. Figure 3.14 shows that the association of Ca^{2+} ions with α_{s1}-casein decreases with decreasing temperature, and the same is true of the other caseins. The loss of CCP presumably causes a weaker attraction between submicelles and possibly a weaker binding of individual casein molecules in a submicelle.

The above-mentioned changes of the micelles cause the milk to obtain other properties. For example, its viscosity increases significantly. The colloidal stability of the casein micelles is definitely greater; consequently, the milk shows poor rennetability during cheese making. All of these changes do not occur immediately on cooling, but they take some 24 h at 4°C before being more or less completed. On subsequent heating of the milk, β-casein returns to the micelles, and the amount of colloidal phosphate increases again. These changes occur slowly at, say, 30°C. Heating the milk briefly to 50°C and cooling it to 30°C reestablishes its original properties, at least as far as rennetability and viscosity are concerned. As mentioned, it is questionable whether the casein micelles have become identical to the original micelles.

3.2.2.3 High Temperature

On increasing the temperature, there is an initial continuation of the trends shown in Figure 3.14. The micelles shrink somewhat and the amount of colloidal phosphate increases. Figure 2.8A shows that the latter change occurs slowly. Moreover, the additional colloidal phosphate may not have the same properties as the natural phosphate.

At temperatures above 70°C the casein molecules become more flexible (below this temperature they are not flexible, but the hairs are), as if part of the submicelle structure melts. At still higher temperatures (above 100°C), dissolution of part of the κ-casein occurs. The extent closely depends on pH; no dissolution occurs below pH 6.2 (measured at room temperature), but there is almost complete dissolution at pH 7.2. The explanation is uncertain. At least part of the

effect must somehow be due to the increased effect of entropy at high temperature. But other factors, such as the absence of serine phosphate in the part of the κ-casein chain that is inside the submicelle, may also be involved. Because of this, κ-casein is weakly or not bound to the colloidal phosphate. Otherwise, micelle-like particles remain at high temperatures, even at 140°C.

If serum proteins are present, as is always the case in milk, another essential change also occurs at high temperature. Serum proteins become largely associated with the casein micelles during their heat denaturation, and they largely become bound to the micelle surface. The association should at least partly be ascribed to formation of —S—S— linkages. An example is the association of β-lactoglobulin with κ-casein. Most of these associations are irreversible on cooling.

3.2.2.4 Acidity

Figure 3.15 illustrates some of the changes that result from a pH decrease. The colloidal phosphate goes into solution, with the dissolution being completed at pH 5.25. Removal of all of the calcium requires a still lower pH, i.e., until below the isoelectric pH of casein. Figure 3.15 further shows that the absolute value of the ζ potential (which results from the net charge of the micelles) decreases by decreasing the pH. This is because of increasing association of hydrogen ions with the acid and basic groups of the protein, but also because of an increasing calcium ion activity, as calcium ions also associate with acid groups. In other words, Ca substitutes calcium phosphate to a certain extent. On further decrease of the pH, the negative charge of casein increases again, due to dissociation of the calcium ions, and eventually decreases again, due to association with H^+ ions. At still lower pH casein becomes positively charged. Furthermore, lowering the pH leads at first to swelling of the micelles and eventually to considerable shrinkage (Fig. 3.15C). When the pH is lowered to, say, 5.3, a large part of the caseins goes into ''solution''; more so with increasing hydrophobicity of the casein concerned. Figure 3.15D refers to a temperature of 20°C; at higher temperatures the effect is smaller, at lower temperatures greater (though then part of the dissolved casein is included in loose submicelles).

The average particle size changes little in the pH region considered. All the same, particles are quite different at high and low pH values. At physiological pH, it is primarily the colloidal calcium phosphate that keeps the micelles intact. When the pH is lowered, the phosphate dissolves, resulting in increasingly weaker bonds. Consequently, swelling of the micelles occurs, along with dissolution of part of the casein. At low pH, internal salt bridges between positive and negative groups on the protein keep the molecules together. Obviously, the total attraction is strongest near the isoelectric pH of casein, i.e., near pH 4.6. We may conclude that the number and/or the strength of the sum of all kinds of bonds is weakest near pH 5.25; this optimum pH depends somewhat on temperature.
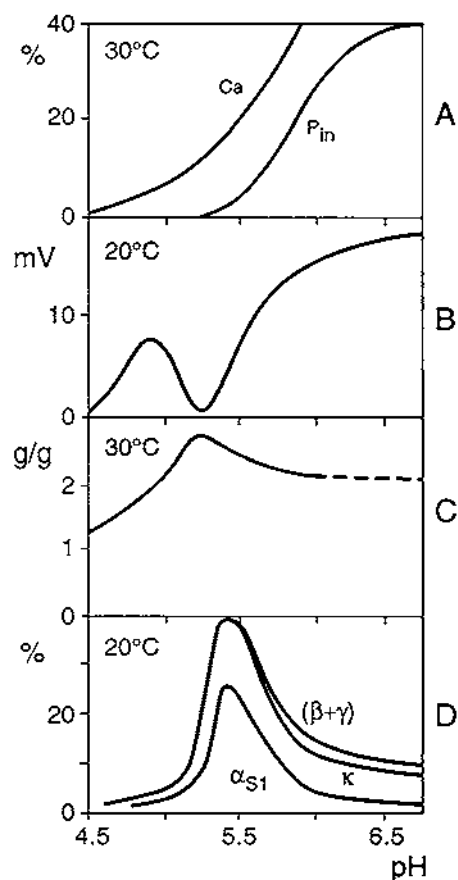
FIGURE 3.15   Properties of casein micelles as a function of pH. (A) Percentage of calcium and inorganic phosphorus in the micelles. (B) Negative electrokinetic or ζ potential. (C) Amount of water per g dry casein in separated micelles. (D) Percentage of the different caseins that cannot be separated by centrifugation (in milk).

Increasing the pH of milk also causes swelling of the micelles and their eventual disintegration. Presumably, the colloidal phosphate passes into another state.

3.2.2.5   Disintegration

Weakening of the bonds between the submicelles or those between protein molecules in the submicelles can lead to disintegration of the micelles. The former

may be due to dissolution of the colloidal phosphate at constant pH, e.g., by adding an excess of a Ca binder like citrate, EDTA, or oxalate. Examples of the effect of various additions are given in Table 2.8. The second type of disintegration occurs by addition of reagents like sodium dodecyl sulfate or large quantities of urea, which break hydrogen bonds and/or hydrophobic interactions (see also Table 2.15). Reagents that break —S—S— linkages do not disintegrate the micelles, but it is not known if less rigorous changes occur.

3.2.3 Colloidal Stability

Casein micelles are colloidal particles, large enough to flocculate as a result of mutual attraction caused by van der Waals forces. Under physiological conditions, however, the micelles do not flocculate. Hence, there must be counteracting repulsive forces that prevent aggregation. The conditions in milk can be altered in such a way that the micelles flocculate, e.g., by adding large quantities of calcium ions or ethanol, or by applying very high temperature. In all such cases, casein micelles are much less stable than dissolved casein because of the much higher entropy of the free molecules. [Flocculation can only occur if it results in a lower free energy, which, in turn, is due to a decrease of enthalpy, i.e., bond energy. Flocculation also causes a decrease of entropy, and this increases the free energy (since $\Delta G = \Delta H - T \Delta S$), and thereby counteracts flocculation. But much of the entropy has already been lost in the formation of the micelles, so that the additional decrease in entropy on aggregation of the micelles is more or less negligible.]

Obviously, the colloidal stability of the casein micelles requires some explanation.

3.2.3.1 Cause of Stability

In the classical DLVO theory, electrostatic repulsion and attractive van der Waals forces are taken into account to explain the colloidal stability of particles. Because of their charge, the micelles have a certain ζ potential (which can be determined). Assuming them to be perfect spheres, the interaction energy can be calculated; this is the free energy needed to bring two micelles from infinite to some close distance from each other. An example is given by curve A in Figure 3.16. At very close distance, particles attract each other (negative free energy), but repulsion often occurs at some larger distance, as curve A shows. If the maximum in the interaction energy is many times kT (= the average heat energy between two particles that encounter each other), very close approach will not be possible. In other words, the free activation energy then would prevent flocculation. Although there are many uncertainties in calculating curves such as A, it is quite clear that the above electrostatic repulsion would not suffice to prevent flocculation of casein micelles.

interaction energy / kT
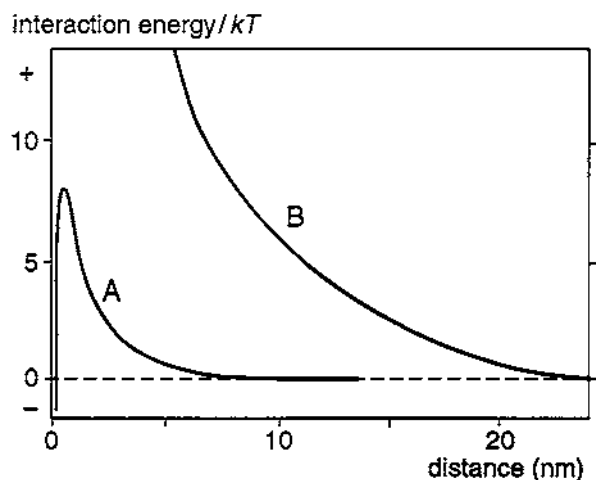
FIGURE 3.16   Approximate representation of the interaction free energy (positive means repulsion) between two casein micelles as a function of distance between the core surfaces of the micelles. (A) Calculated according to the DLVO theory. (B) Rough estimate for steric repulsion + van der Waals attraction.

An additional mechanism for providing colloidal stability must therefore be present. Steric repulsion caused by the hairy layer around the micelles must be responsible. A hair in such a layer consists of a flexible peptide chain, which keeps exhibiting conformational changes because of Brownian motion. If the presence of another particle restricts the freedom of motion of the hair, this causes loss of entropy and thus repulsion. This is called *volume restriction*. It yields one of the terms in the formula for steric repulsion. The other term is the so-called *mixing* term, which becomes important if the hairy layers of two micelles overlap. If the solvent quality of the liquid for the hairs is poor, mixing will lead to attraction of the micelles; if it is good, there will be repulsion. The parts of the κ-casein sticking out from the micelles readily dissolve in milk serum. This means that the solvent quality is good. Thus there is repulsion, which will monotonously increase with closer approach of the surfaces of the micelles, because of increasing interpenetration of the hairy layers. The repulsion energy must be considerable, though its magnitude cannot be calculated. Curve B in Figure 3.16 gives a schematic trend.

Steric repulsion cannot explain all observations. To begin with, if the interaction curve B in Figure 3.16 is taken as true, micelles would never flocculate

(unless they lose their hairy layer). Moreover, some variables that considerably affect micelle stability can hardly have an effect on steric repulsion. Examples are increase of the calcium ion activity, decrease of the pH, and decrease of the dielectric constant, all of which would cause a decrease of electrostatic repulsion and are observed to enhance flocculation. The following model gives a hypothetical explanation.

3.2.3.2 A Model

If two micelles closely approach one another due to Brownian motion, the hairy layers of the micelles may overlap. Only by overlapping can steric repulsion occur. The hydrodynamic thickness of each layer is approximately 7 nm. Therefore, at least some of the hairs protrude over a greater distance from the core surface of the micelles. This means that repulsion will occur at a distance of around 20 nm. As is shown in curve A of Figure 3.16, at this distance any electrostatic repulsion resulting from charged groups on the micelle surface would be negligible. This follows from the small thickness of the electrical double layer in milk, i.e., about 1.1 nm (Section 2.2.2.1). But not all of the charge is on the core surface of the micelles; a considerable part is on the hairs, as is schematically shown in Figure 3.17a. (The situation is in fact more complicated than depicted because there are more charges on the hairs, including positive ones.) It implies that the electrostatic repulsion extends over a distance much farther from the core surface of the micelles. Though not large enough in itself to prevent flocculation, the electrostatic repulsion can affect the distance of closest approach of the micelles. Variation in the electrostatic charge therefore has an effect on the extent of interpenetration of the hairy layers. This is diagrammatically shown in Figure 3.17b.

Hairs of both micelles will frequently touch one another if the hairy layers overlap during an encounter. Crosslinking between reactive sites may occur if such sites happen to touch. The tendency to form linkages presumably increases with increasing interpenetration depth of the hairy layers. Micelles may be linked together by simple salt bridging, Ca bridging, formation of colloidal phosphate linkages, or, at high temperature, formation of covalent linkages between amino acid residues (Table 6.2). When it concerns noncovalent bonds that may be very short-lived, like Ca bridges, numerous bonds presumably must be simultaneously formed between two micelles for the contact to be lasting.

3.2.3.3 Causes of Instability

Although native casein micelles are stable, a change in environment may lead to flocculation or, in more general terms, aggregation of the micelles. The main causes for aggregation are listed in Table 3.7. Generally, the change in conditions results in changes in the micelles before aggregation occurs. Furthermore, the aggregation mostly seems irreversible. The various cases will be briefly dis-
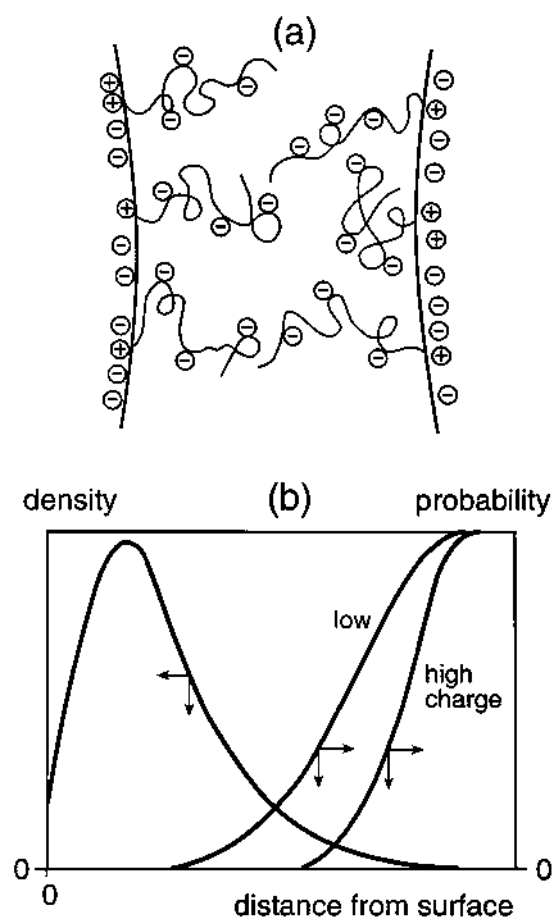
FIGURE 3.17 Hypothetical picture of interactions between two casein micelles. (a) Overlapping hairy layers and electrostatic charges. (b) Average density of material of the protruding peptide chains of the left micelle as a function of the distance from the core surface of that micelle, as well as the relative probability of finding a segment of a hair of the right micelle in the hairy layer of the left one, for a low and a high net charge of the hairs, respectively.

TABLE 3.7   Various Causes for the Aggregation of Casein Micelles

Cause	Micelles changed?	Aggregation reversible?	Aggregation at low temperatures?
Long storage (age gelation)	Yes	No	No
At air–water interface	Spreading	No	No
High temperature (heat coagulation)	Chemically	No	—
Acid to pH ≈ 4.6	No CCP left	(Yes)[1]	No
Ethanol	Presumably	No	?
Renneting	κ-Casein split	No	No
Excess Ca^{2+}	More CCP	Yes	?
Freezing plus thawing	Presumably	(Yes)[2]	—
Addition of some polymers	No	Mostly	Yes

[1] At neutral pH, the aggregates dissolve again but the natural micelles do not reappear.
[2] Partly, depending on conditions.
CCP, colloidal calcium phosphate.

cussed. Heat coagulation and renneting are dealt with in Sections 6.2 and 21.3, respectively.

Age thickening and gelation mainly occur in evaporated and sweetened condensed milk. The explanation is unclear. Electron microscopy reveals that the micelles in these products become much less smooth and increasingly show protrusions; see also Figure 16.7. This change causes an increase of the viscosity of the product and eventual formation of a continuous network, hence a gel.

Beating in of air in milk causes adsorption of casein micelles onto the air bubbles formed. The micelles can partly spread over the bubble surface. After the air itself has dissolved, the adsorbed material remains behind as a kind of bag (deflated balloon), in which the micelles can be recognized. This so-called ghost membrane is very stable, which implies that the casein micelles remain aggregated. The molecular explanation of the phenomenon is uncertain; the partly spread micelles can conceivably touch each other at sites devoid of hairs, leading to their fusion.

Aggregation as caused by acidification can simply be explained: casein becomes insoluble near its isoelectric pH. Note that even a slight lowering of the pH results in a significantly decreased charge (partly because of increased calcium ion activity), which in itself diminishes colloidal stability.

Addition of ethanol lowers the solvent quality for the hairs of κ-casein. This causes the hairy layer to collapse, and the steric repulsion to diminish or even to change in attraction. The latter effect is enhanced by a decrease of the dielectric constant, resulting in a reduced electrostatic repulsion. Moreover, the

colloidal phosphate passes into another, unknown state which causes aggregation of the ''micelles'' to be irreversible. The lower the pH of the milk, the smaller the ethanol concentration needed to cause coagulation. This principle may be applied to quickly detect slight sourness in milk. In the so-called alcohol stability test, milk and ethanol are mixed in fixed proportion. If visible flocculation occurs, the milk is taken to be sour.

An excess of Ca^{2+} ions enhances the possibilities of Ca bridge formation. Moreover, it decreases the charge of the micelles and increases the supersaturation with respect to calcium phosphate in the milk serum. The latter would cause formation of additional colloidal phosphate (presumably, the serine phosphate residue of the protruding part of κ-casein can take part in it), which would cause fusion of micelles. In other words, if much CCP can be deposited, the steric repulsion is overcome, leading to aggregation. Occasionally, this seems to occur in fresh milk that spontaneously curdles just after milking. This so-called Utrecht milk abnormality is related to a low citrate content and an ensuing high calcium ion activity in the milk.

Freezing of milk leads to highly increased salt concentrations in the remaining nonfrozen solution. The situation is comparable to that in the preceding paragraph, but here there also is true salting-out. Slow changes occurring in the CCP cause the aggregated micelles to be not fully redispersible after thawing (see also Section 10.2).

The last mentioned cause for aggregation (addition of polymers) is of a completely different nature. Some long-chain polymer molecules can adsorb onto casein micelles. If the polymer tends to form a network, the micelles are incorporated into it. For example, a weak gel is obtained by adding a little κ-carrageenan to chocolate milk, thereby avoiding sedimentation of cocoa particles. Higher concentrations of suitable polymers may cause a kind of coprecipitation of polymer and casein. Presumably, the micelles remain virtually unaltered. Therefore, the aggregation concerned is reversible.

3.2.3.4   *Effect of Temperature*

The lower the temperature, the higher the colloidal stability of casein micelles. Table 3.7 shows that most aggregation reactions do not occur at low temperature, e.g., 5°C. That does not imply that no strong bonds between micelles can exist at low temperature. After their aggregation at, for instance, 30°C by renneting or acidification of milk, the formed gel is not dispersed again on lowering the temperature to, say, 5°C; it even becomes firmer, which implies that the number or the strength of the bonds between the micelles increases. In other words, the casein micelles do not flocculate at low temperature because of a high activation free energy for flocculation. This may be explained as follows. Figure 3.14 shows that lowering the temperature increases the voluminosity of the micelles. This can partly be ascribed to formation of a thicker hairy layer or of a layer of another

composition; β-casein chains especially may protrude from the micelle surface at low temperature. Because of this, steric repulsion would be stronger, and it then also remains after removal of the hairs of κ-casein, as occurs on renneting. Apart from that, swelling of the micelles results in a smaller van der Waals attraction.

At high temperatures micelle stability decreases, and it decreases more so at a lower pH. This is discussed in Section 6.2.

3.2.3.5   Consequences of Aggregation

Casein micelles encounter each other frequently because of Brownian motion. After making contact they will draw apart again, but sometimes they can keep together due to formed bonds and become aggregated. This is shown schematically in Figure 3.18, reaction 1. Subsequently, fusion of micelles may occur as is shown in reaction 2. Usually, fusion will be a slow process, but it is quicker if most of the hairs have been removed. Essentially, fusion is the same reaction as the formation of casein micelles from submicelles. As is discussed in Section 3.2.2 (Fig. 3.13), there is a dynamic equilibrium between submicelles and micelles. Such an equilibrium will likewise be established when two micelles have
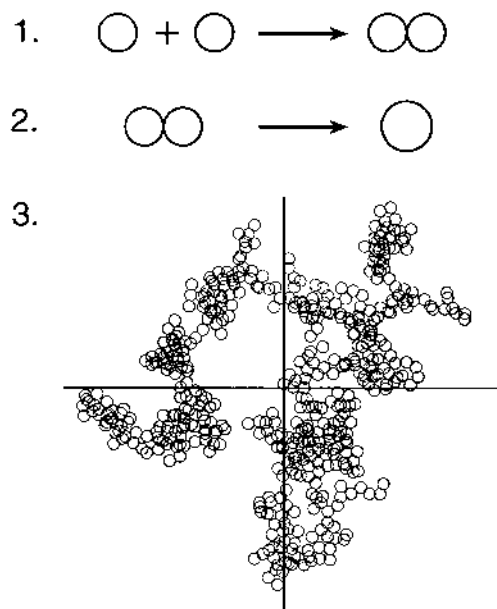
FIGURE 3.18   Aggregation of casein micelles. 1. Flocculation reaction. 2. Fusion. 3. Example of a floc of micelles formed during ongoing flocculation.

become one particle due to aggregation. Fusion then results in formation of particles of roughly spherical shape.

Most aggregations need a much longer time for fusion (reaction 2) than for flocculation (reaction 1), and run as follows: Small flocs first flocculate with each other and with single micelles, and then increasingly larger flocs flocculate with each other. Due to the chance factor in flocculation, the flocs formed have a quite open (rarefied) structure as is shown in Figure 3.18, item 3. Such flocs are of a fractal nature, i.e., they are scale-invariant. That means that the number of particles in a floc of radius R is proportional to R^D, where D is the fractal dimensionality, which is always <3. For flocculating casein micelles it has generally been found that $D \approx 2.3$. Because the volume occupied by a floc is proportional to R^3, the volume fraction of particles in the floc (ϕ_{floc}) is proportional to R^{D-3}. Thus, the floc becomes ever less dense as it grows in size; ϕ_{floc} is inversely proportional to $R^{0.7}$. As soon as the average ϕ_{floc} becomes equal to the volume fraction of particles in the liquid (ϕ), the flocs fill the total volume and a gel forms.

If reaction 2 is rapid compared to reaction 1, large and dense particles emerge and eventually a precipitate forms. A precipitate is also obtained when formed flocs show a strong tendency to contract or synerese (Section 21.3), or when the liquid is stirred during flocculation.

There are also other mechanisms of gel formation, as described in relation to ''age gelation'' and ''polymers'' in Table 3.7.

3.2.3.6 Kinetic Aspects

Smoluchovski's theory gives the encounter frequency of particles in Brownian motion. Assuming a certain part of the encounters to lead to flocculation and counting two particles that are flocculated as one, the decrease of the number (N) of particles per unit volume with time (t) would be given by

$$-dN/dt = (4\, kT\, N^2/3\eta_c)/W \tag{3.2}$$

where k is Boltzmann's constant, T is absolute temperature, η_c is viscosity of the continuous phase, and W is the stability factor, i.e., the ratio of the number of encounters to the number leading to lasting contact. In principle, W can be calculated from colloidal-stability theory, though for casein micelles it usually cannot. Flocculation causes the average original particle radius (a) to increase. In case of ''fractal'' flocculation, the radius increases rapidly, as has been outlined above. Combining fractal flocculation theory with Eq. (3.2), integration of (3.2) yields the gelation time (t_{gel}), which turns out to be

$$t_{gel} = (\pi a^3 \eta_c / kT)\, \phi^{3/(D-3)}\, W \tag{3.3}$$

where a is the radius of the casein micelles. Obviously, gelation time greatly depends on ϕ (total volume fraction of particles in the liquid). For example, for

$\phi = 0.1$ the gelation time turns out to be 110 times as long as for $\phi = 0.3$. This is the main cause for the great difference in, for instance, heat coagulation time of evaporated as compared to nonevaporated milk.

What magnitude of the stability factor W is needed to provide stability? From Eq. (3.3), t_{gel} is found to be <10 s for skim milk, for $W = 1$. But skim milk, if sterile and devoid of proteolytic enzymes, can be kept at room temperature for at least 3 years without the casein micelles aggregating to a gel. This implies that W is at least 10^7. In other words, two encountering micelles very rarely make lasting contact.

If reaction 2 (Figure 3.18) is faster than reaction 1, formation of a gel does not occur, but formed particles increase in size. The moment at which visible particles appear may be considered the coagulation point. That time depends much less on ϕ, and is much longer than the corresponding t_{gel} in Eq. (3.3). This is because the encounter frequency of particles in Brownian motion decreases strongly as the particles increase in size, hence decrease in number [see Eq. (3.2)]. In practice, however, slight shear rates caused by, for instance, small temperature fluctuations would strongly enhance aggregation rate as soon as the aggregates become large (several μm). The theory concerned will not be discussed.

3.3 PHYSICAL PROPERTIES

Some of the physical properties of milk are to a considerable degree determined by its being a dispersion of colloidal particles. This is obvious for optical properties because milk is turbid (see Fig. 1.1). Rheological properties are also strongly dependent on concentration and properties of the particles in milk.

3.3.1 Optical Properties

The *refractive index*, n, of a transparent liquid is defined as the ratio of the velocity of light in air to that in this liquid. It depends on the wavelength of the light and decreases with increasing temperature. It is generally determined at a wavelength of 589.3 nm and at 20°C.

The refractive index of milk (about 1.338) is determined by that of water (1.3330) and the dissolved substances. Particles larger than about 0.1 μm do not contribute to n. Consequently, fat globules, air bubbles, or lactose crystals do not contribute to the refractive index of milk and milk products, though they may hamper the determination of n, by the turbidity they cause. Casein micelles, though many are larger than 0.1 μm, do contribute to n because they are inhomogeneous (consisting of far smaller building blocks) and have no sharp boundary.

The difference between n of water and an aqueous solution is given by

$$n(\text{solution}) - n(\text{water}) = \Delta n \approx \rho \sum m_i r_i \qquad (3.4)$$

where ρ is the mass density of the solution, m is mass fraction of a solute, and r is its specific refraction increment. The equation is not precise because the contribution of the various components (i) is not always precisely additive. Values for r (at 589.3 nm and 20°C) are, in ml/g (which implies that ρ has to be in g/ml):

> Casein micelles, 0.207 (expressed per g casein)
> Serum proteins, 0.187
> Lactose, 0.140
> Other dissolved milk components, ~0.170
> Sucrose, 0.141

With these data, the refractive index of milk products can be calculated. When concentrating milk or another liquid by evaporation, $\Delta n/\rho$ increases proportionally with the concentration factor. Since n can be determined easily, rapidly, and accurately (standard deviation 10^{-4} or better), it is a useful parameter to check changes in composition, such as solids-not-fat content.

Light scattering is caused by particles whose refractive index differs from that of the surrounding medium. For instance, $n_{fat\ globule}/n_{plasma} \approx 1.084$. Figure 3.19 gives some examples of light scattering as caused by fat globules. Because the fat globules in one sample of milk vary considerably in size, mean curves should be calculated. From results as presented in Figure 3.19, particle size or fat content can be derived through turbidity measurements on strongly diluted samples. The casein micelles also scatter light, though far less than the fat globules do. This
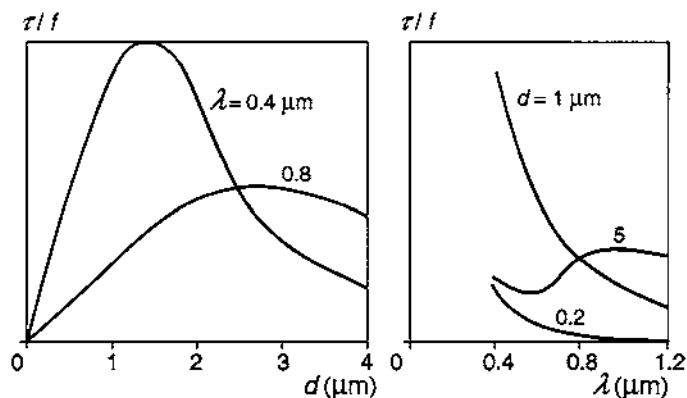
FIGURE 3.19 Light scattering by milk fat globules. Total turbidity (τ) per percentage of fat (f) as a function of globule diameter (d) and wavelength (λ). Globules should be of uniform size and the suspension highly dilute.

is because they are smaller in size and inhomogeneous; furthermore, the difference in refractive index with the solution is somewhat smaller.

In the ultraviolet, plasma as well as fat (double bonds) strongly absorb light, especially for wavelengths <300 nm. In the near-infrared, numerous strong water absorption bands occur, but there are also some absorption bands that can be used to estimate the contents of fat, protein, and lactose in milk.

3.3.2 Viscosity (η)

Under most conditions milk behaves as a Newtonian liquid; this means that the shear stress is proportional to the shear rate (dv/dx). The viscosity of milk is about twice that of water. The difference is caused by the dissolved substances and the dispersed particles. Viscosity of a dispersion usually is well described by the semiempirical Eilers equation:

$$\eta \approx \eta_0 \left(\frac{1 + 1.25\phi}{1 - \phi/\phi_{max}} \right)^2 \tag{3.5}$$

where η_0 is the viscosity of the solvent and ϕ is the volume fraction of the dispersed particles; all particles that are significantly larger than the molecules of the solvent (i.e., water) must be considered. This means that even lactose molecules should be included. ϕ pertains to the hydrodynamic volume of the particles, which includes hydration water, hairy layers, and cavities. Thus we have for milk:

$$\phi \approx \phi_f + \phi_c + \phi_s + \phi_l \tag{3.6}$$

where ϕ_f relates to fat globules, whose volume is ~1.11 ml · g^{-1} of fat; ϕ_c relates to casein micelles (~3.9 ml · g^{-1} of casein); ϕ_s relates to serum proteins (~1.5 ml · g^{-1} of protein); ϕ_l relates to lactose (~1 ml · g^{-1} of lactose).

ϕ_{max} is the hypothetical volume fraction that would cause all of the particles to touch each other, leading to infinite η. In milk ϕ_{max} is high, presumably about 0.9, because the particles vary widely in size. For milk, $\eta_0 \approx 1.02\eta_{water}$. The increase as compared to η_{water} is caused by dissolved salts, etc.

The influence of the fat globules is shown in Figure 3.20A. Often the behavior of the liquid remains Newtonian up to $\phi_f \approx 0.4$, unless dv/dx is quite low. In raw cream or milk of low temperature, agglutination of fat globules occurs (see Section 3.1.4.2). It causes η to increase and to become dependent on dv/dx. Figure 3.20C gives examples; the liquid is shear rate thinning. Such behavior implies that the liquid has an apparent viscosity (η'). At higher fat contents, η' depends even more closely on dv/dx. Cream of >40% fat always shows non-Newtonian behavior.

The casein micelles contribute significantly to the value of η (Fig. 3.20D) because of their high voluminosity (Section 3.2.1.2). Compare also both curves in Figure 3.20B. For the UF-concentrated skim milk, casein micelles make up a
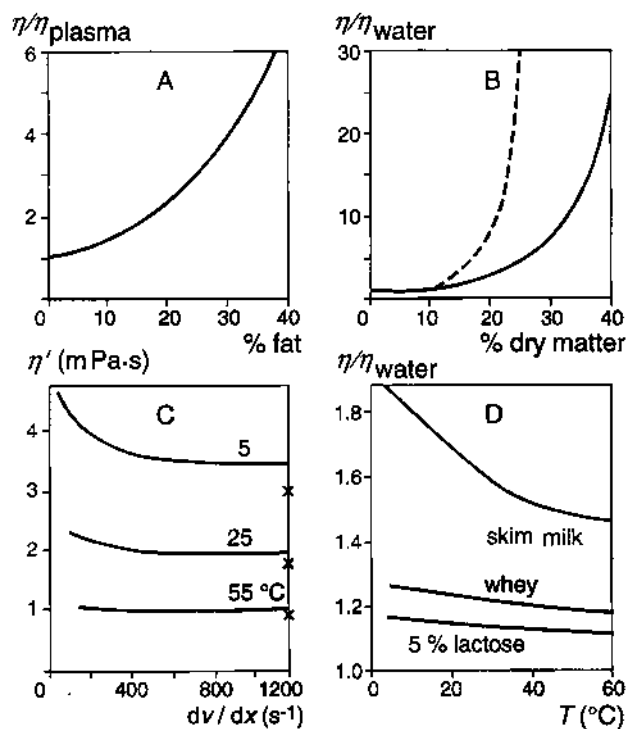
FIGURE 3.20   Influence of some variables on viscosity (η). (A) Milk and cream at 40°C. (B) Skim milk and concentrated skim milk at 20°C; the broken line refers to ultrafiltered skim milk. (C) Raw milk of 5% fat; dv/dx = shear rate; η' = apparent viscosity; crosses indicate 2 × η$_{water}$. (D) Influence of measuring temperature (T).

far greater part of the mass for a given percentage dry matter than is the case for evaporated skim milk. The contribution of the micelles appears to closely depend on temperature (Fig. 3.20D). At low temperature, the voluminosity of the micelles is markedly increased and part of the β-casein becomes dissociated from the micelles. Consequently, the viscosity increases steeply.

Obviously, temperature affects viscosity in a variety of ways. Heat treatment of skim milk to such a degree that the serum proteins become insoluble causes an increase in viscosity by about 10%. This may be explained by increase of the voluminosity of the serum proteins.

Increasing the pH of milk also increases its viscosity, presumably by additional swelling of casein micelles. Slightly decreasing the pH usually leads to a

small decrease of η. A more drastic pH decrease causes η to increase, which is due to aggregation of casein. Homogenization of milk has little effect on η, but homogenization of cream may considerably enhance apparent viscosity (see Section 8.7). Further data are given in Table 3.4.

SUGGESTED LITERATURE

- A short introduction on colloidal aspects in foods is given by:
  P. Walstra, in: O.R. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, Chapter 3, pp. 95–156.
- Much more comprehensive and deeper, but slightly outdated in some respects, is:
  E. Dickinson and G. Stainsby, *Colloids in Foods*, Applied Science, London, 1982.
- Aspects of milk fat globules are discussed in:
  P.F. Fox, ed., *Advanced Dairy Chemistry*, Vol. 2, *Lipids*, 2nd ed. Chapman and Hall, London, 1995, especially Chapters 3 (fat globule synthesis), 4 (physicochemical aspects) and 7 (lipolysis).
- Far more comprehensive, but partly out of date, is:
  H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.
- Lipolysis of milk is discussed in an IDF report:
  Flavour impairment of milk and milk products due to lipolysis, International Dairy Federation, Document 118, Brussels, 1980.
- as well as in:
  H.C. Deeth and C.H. Fitz-Gerald, Lipolytic enzymes and hydrolytic rancidity in milk and milk products, Chapter 7 in: P.F. Fox, ed., *Advanced Dairy Chemistry*, Vol. 2, *Lipids*, 2nd ed., Chapman and Hall, London, 1995.
- Casein micelles are discussed in:
  P.F. Fox, ed., *Advanced Dairy Chemistry*, Vol. 1, *Proteins*, Elsevier, London, 1992, especially Chapters 3 (casein micelles), 14 (renneting), and 15–17 (stability).
- Several physical properties of milk are discussed by:
  J.W. Sherbon, in: N.P. Wong, ed., *Fundamentals of Dairy Chemistry*, 3rd ed., Van Nostrand Reinhold, New York, 1988, Chapter 8.

4

Microbiology of Milk

Milk must have a desirable chemical composition and must be of satisfactory hygienic quality. This is essential in terms of public health, the quality of the products made from milk, and the suitability of milk for processing. Components that are foreign to milk, but enter the milk in the udder or during or after milking, as well as any changes occurring in the milk are often detrimental to its quality. These matters are the subject of milk hygiene. Microbial, chemical, and physical hygiene may be distinguished. Examples are microorganisms that may produce a health hazard (food infection or food poisoning) or that spoil the milk, e.g., because they turn it sour during storage. Light-induced off-flavors, fat oxidation, and fat hydrolysis result from chemical or enzymic transformations. Furthermore, compounds that are potentially harmful to the consumer, such as antibiotics, disinfectants, pesticides, and heavy metals, can enter the milk.

In this chapter, microbiological aspects of milk hygiene are discussed. Chemical contamination of milk is covered in Section 2.6.2.

4.1 GENERAL ASPECTS

Milk is a good source of nutrients and edible energy, not only for mammals but for numerous microorganisms, which thus can grow in milk. It primarily concerns bacteria, but some molds and yeasts can also grow in milk. In this section, some general aspects of growth and inhibition of microbes in milk will be discussed.

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4.1.1   Growth

Bacteria multiply by division. Every cell division yields two new bacterial cells. The multiplication is a geometrical progression $2^0 \rightarrow 2^1 \rightarrow 2^2 \rightarrow 2^3 \ldots \rightarrow 2^n$. If a growing bacterial culture contains N_0 cells ml^{-1}, the bacterial count N after n divisions is

$$N = N_0 \cdot 2^n \qquad\qquad (4.1)$$

or

$$\log N = \log N_0 + n \log 2 = \log N_0 + 0.3\, n \qquad\qquad (4.1a)$$

The time needed for a full cell division thus determines the growth rate. It is called the generation time g; it can be derived from the number of divisions occurring during a certain time t:

$$g = t/n \qquad\qquad (4.2)$$

Consequently, in well-defined conditions the count N after a storage time t can be calculated from Eqs. (4.1a) and (4.2), if N_0 and g are known:

$$\log N = \log N_0 + 0.3\, t/g \qquad\qquad (4.3)$$

Generation time g depends on several factors. In milk, the bacterium species (or strain) and the temperature are of special importance. Other factors involved are pH, oxygen pressure, and concentrations of inhibitors and nutrients, which are all fairly constant in raw milk.

Growth of bacteria thus means an increase in the number present. Current methods for determination generally give the number of colony-forming units (CFU) per ml. Since several bacteria tend to remain attached to each other after division, forming shorter or longer chains of individual cells (up to about 100, but generally less), the colony count may be much smaller than the actual numbers of living cells present. This is especially true for *Lactococcus* and *Streptococcus* species, as well as for some species of *Lactobacillus*. The equations given above should thus be interpreted with care. In some cases, determination of the biomass of bacteria present would be preferable. It should further be realized that yeast cells are far bigger than most bacteria and can produce more metabolites per cell per unit time than bacteria. The same holds true for molds, where the difference between CFU and individual cell number also may differ greatly.

The above equations apply to the exponential growth phase of the bacteria (sometimes called logarithmic or log phase). Figure 4.1 illustrates the various growth phases that can be distinguished. During the lag phase the bacteria do not multiply, primarily because their enzyme system needs adaptation, enabling the bacteria to metabolize the nutrients in the medium. The duration of the lag phase closely depends on the physiological state of the bacteria, the temperature,
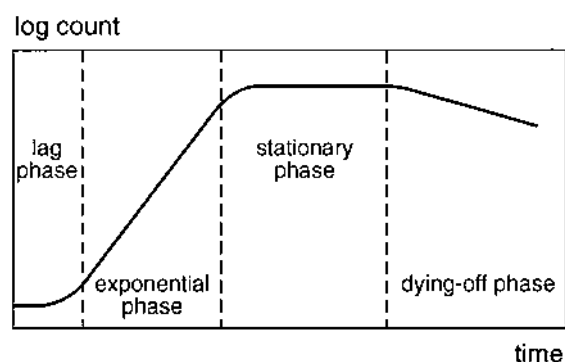
FIGURE 4.1 Growth curve of a bacterial culture.

and the properties of the medium. During the exponential phase, the growth is at a maximum rate until the stationary phase is reached. In the latter phase, some growth still occurs, together with dying off. The decrease of the growth rate is usually caused by action of inhibitors formed by the bacteria themselves and/or by a lack of available nutrients. Eventually, the stationary phase turns into the dying-off phase, during which the count decreases. The former two phases are of special importance for the quality and the keeping quality of milk. In fermented milk products the latter two phases also are essential.

Temperature has a large effect on bacterial growth. Lowering of the temperature retards the rate of nearly all processes in the cell, thereby slowing down growth and decreasing fermentation rate (e.g., acid production). Moreover, it extends the duration of effectiveness of some of the natural bacterial inhibitors in milk. Furthermore, many bacteria coming from a medium such as dung or teat surface and entering a substrate like milk must adapt themselves to the new medium, hence the lag phase. At a lower temperature the lag phase thus will last longer. The extent to which a lowering of the temperature affects bacterial growth depends on the type of organisms present.

Table 4.1 gives some examples of the effect of temperature on generation time. It shows that lactic acid bacteria will not spoil cold-stored milk and that at 30°C pseudomonads grow more slowly than other bacteria. The temperature dependence of the growth rate has consequences for the keeping quality of milk, as is shown in Table 4.2. At high storage temperatures, the milk has a poor keeping quality, even if its initial count is low; it should be processed within a few hours after production.

Figure 4.2 shows that a low initial count and a low storage temperature are essential. Whether the milk is kept at a low or at a higher temperature, a

TABLE 4.1 Generation Time (h) of Some Groups of Bacteria[1] in Milk (Not Including the Lag Phase)[2]

Temperature (°C)	5	15	30
Lactic acid bacteria	>20	2.1	0.5
Pseudomonads	4	1.9	0.7
Coliforms	8	1.7	0.45
Heat-resistant streptococci	>20	3.5	0.5
Aerobic sporeformers	18	1.9	0.45

[1] Within these groups of bacteria, generation time varies widely among species and strains. The values as mentioned hold for the fastest growing representatives at the given temperatures.
[2] Approximate examples.

lower initial count always means that it takes more time for the milk to spoil. Naturally, the combination of low initial count and low storage temperature is to be preferred. It is important to note that for raw milk to be kept for several days, the type of contamination may be of greater importance than the total count. For example, contamination by 10^5 mastitis bacteria per ml of milk has less effect on the keeping quality at low temperature than 10^3 psychrotrophs per ml. These aspects are further illustrated in Figure 4.3.

Growth of microorganisms in raw milk is generally undesirable. Of all treatments known to reduce the growth of microorganisms, for raw milk only

TABLE 4.2 Approximate Example of the Effect of the Keeping Temperature of Milk on Its Count After 24 h, and on Its Keeping Quality (Initial Count 2.3×10^3 ml^{-1})

Milk held at (°C)	Count after 24 h (ml^{-1})	Keeping quality[1] (h)
4	2.5×10^3	>75
10	1.2×10^4	30
16	1.8×10^5	19
20	4.5×10^6	11
30	1.4×10^9	5

[1] Keeping quality is defined here as the storage time during which the milk remains suitable for processing (count not exceeding $0.5–1.0 \times 10^6$ ml^{-1}).
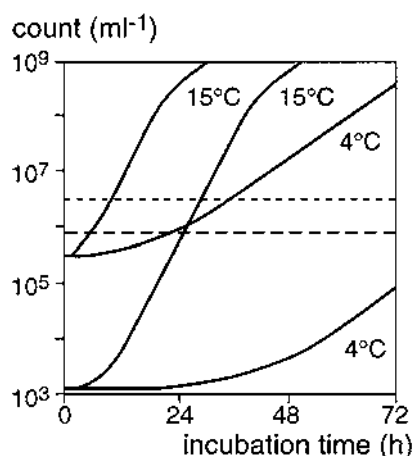
FIGURE 4.2 Change of the colony count during the keeping of milk of two initial counts, at two temperatures. Approximate examples. Broken lines mark the region where spoilage of milk usually becomes observable.

lowering of the temperature is generally feasible. Heat treatment kills bacteria (Section 6.3.3). Milk contains some natural growth inhibitors (Section 4.1.2), but addition of a bacterial inhibitor to milk generally is not allowed by the public health authorities. Such inhibitors may pose a health hazard or cause off-flavor development. But in some tropical countries high temperatures and poor hygienic standards prevail; in order to bring the milk in good condition to where and when it is needed for processing, addition of a bacterial inhibitor seems unavoidable. The use of hydrogen peroxide can under certain conditions be tolerated. A better preservation may result from activation of the lactoperoxidase–thiocyanate–H_2O_2 system (Section 4.1.2).

4.1.2 Milk as a Substrate for Bacteria[1]

This section discusses the influence of the properties of milk and milk products on growth and metabolic action of bacteria. This is to some extent an oversimplification because milk products are ecosystems. In other words, the interaction between bacteria and environment determines what will happen; bacterial action affects the environment, and the latter determines which bacteria can proliferate. The environment includes the properties of the substrate (i.e., milk or a deriva-

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[1] This section was for the most part taken from P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (1984), Section 11.6, by permission of John Wiley and Sons, New York.

storage time (days)
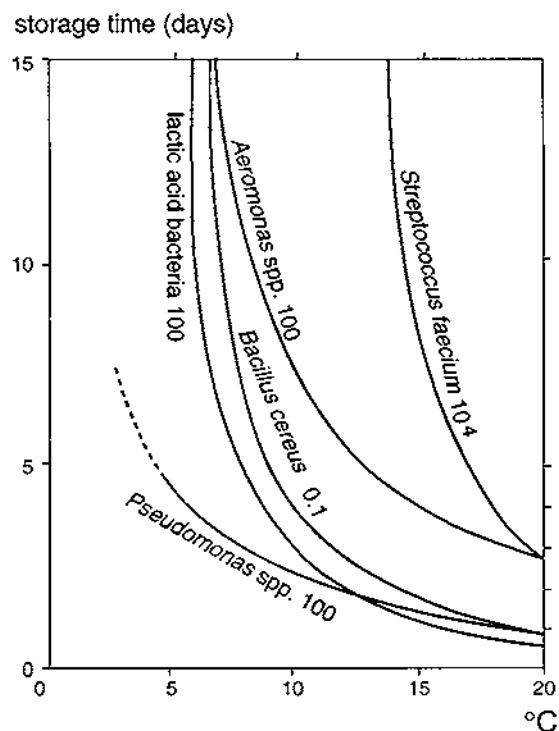
FIGURE 4.3   Time needed to reach a count of 10^6 bacteria per ml when keeping raw milk at various temperatures. The bacterium considered and its initial count (in ml^{-1}) are indicated. Approximate examples, only meant to illustrate trends.

tive) and outside conditions, of which temperature is by far the most important variable. The bacterial population can vary greatly. Raw milk left in contact with the outside world is essentially an open ecosystem; almost any bacterium can be present, and the properties of the milk and the temperature largely determine which bacteria will outmatch the others. Many milk products are closed or controlled ecosystems, and the microbial changes occurring depend greatly on the particular contamination by bacteria that has occurred. Not only are species and numbers of bacteria important, but so are their physiological condition, such as stage of growth (Fig. 4.1), and the possible presence of bacteriophages (Section 11.3). The effect of the environment is usually different for growth, fermentation, and, in the case of certain species, sporulation. Generally, conditions permitting growth are more restricted than those for fermentation. For instance, several lactic

acid bacteria do not grow to any extent near 5°C but, other conditions being favorable, they may go on producing lactic acid from lactose. Conditions inducing sporulation, and whence possible growth and fermentation of the ensuing bacteria, are usually rather restricted.

Milk contains such a wide range of nutrients, including all of the vitamins, that numerous species of bacteria find sufficient raw material for fermentation and growth. The result is well known: Raw milk spoils rapidly at room temperature. But because the bacteria that can grow in milk may have very different properties, we should be cautious in applying general rules. For some bacteria, lactose is not a suitable energy source. Others rely on free amino acids as a nitrogen source, and fresh milk contains only tiny amounts of amino acids. Consequently, such bacteria often start to grow after other bacteria have hydrolyzed proteins, thus providing suitable nutrients. Another such example is the production of CO_2 by some lactic streptococci, which stimulates growth of some lactobacilli. (On the other hand, some gram-negative bacteria are inhibited by CO_2.) Some bacteria need specific trace components for growth or fermentation that are missing or present in insufficient concentrations.

Some conditions in milk may be unfavorable for growth of some bacteria. A possible lack of nutrients has been mentioned already. Water activity and ionic strength of milk are never limiting, and pH is so only for a few organisms. But redox potential and O_2 pressure are mostly such that strictly anaerobic bacteria cannot grow. The growth of aerobic bacteria also depends on location; in a cream layer O_2 pressure may be much higher than near the bottom of a deep vessel of milk. At room temperature, bacterial action usually lowers both O_2 level and pH. For very few microorganisms are conditions in milk so unfavorable as to kill them.

Milk contains natural *inhibitors*. Some bacteria do not grow in milk despite the presence of sufficient nutrients and suitable conditions. A mere delay (or lag phase) in growth after addition of the bacteria to the milk is not proof of inhibition; the bacteria may not be adapted to milk (i.e., they have to change their enzyme system before they can use the nutrients available).

An important class of inhibitors is the immunoglobulins (Section 2.4.3), which are antibodies against specific antigens, often bacteria. Thus they are specific for the species and strains of bacteria encountered by the cow, and other bacteria are not inhibited. Mixed milk may contain immunoglobulins active against a wide variety of bacteria, but the concentration is usually low. Though examples of inhibition by IgG and IgA (probably in cooperation with ''complement'') in milk are known, the agglutinative action of IgM is most conspicuous. There is an agglutinin acting against strains of *Streptococcus pyogenes*, another against strains of *Lactococcus lactis* ssp. *lactis* and *cremoris*; generally, *Bacillus cereus* also exhibits agglutination in raw or low-pasteurized milk.

Agglutination means that bacteria meet each other owing to their Brownian

motion and are kept together in (large) floccules by the sticking action of the agglutinin. The floccules can become so large that they sediment, removing the bacteria from most of the milk. Bacteria also can become attached by agglutinins to fat globules if the temperature is low. The cold agglutination and rapid creaming of the fat globules (Section 3.1.4.2) then cause rapid removal of bacteria from most of the milk. The organisms are not killed but rather are restricted in growth because of the depletion of nutrients and the accumulation of inhibiting metabolites in the sedimented layer of floccules. Those concentrated in the cream layer also may be inhibited by the higher O_2 pressure. If agglutination is hindered mechanically, e.g., when a milk is renneted directly after adding a starter (the baceria now become enclosed in the meshes of the paracaseinate network), inhibition is insignificant. The content of agglutinins in milk is highly variable; usually, colostrum has relatively high concentrations.

Some nonspecific bacterial inhibitors are lysozyme and lactoferrin, but their concentrations in cows' milk are so low as to have little effect; human milk contains much more. Lysozyme is an enzyme (EC 3.2.1.17) that hydrolyzes polysaccharides of the bacterial cell wall, particularly splitting off N-acetylmuramic acid; this may cause lysis of the bacteria. Lactoferrin binds Fe, thus reducing the activity of Fe^{2+} ions, which are needed by several bacteria. Possibly some fragments of lactoferrin produced by proteolysis exhibit other antimicrobial activities.

The most important nonspecific inhibitor of milk is the peroxidase–thiocyanate–H_2O_2 system, which is also quite active in saliva. The milk enzyme lactoperoxidase (Section 2.5.2.1) as such does not cause inhibition, but it catalyzes the oxidation of SCN^- by H_2O_2, and one of the intermediates ($OSCN^-$?) is a powerful bacteria killer. Milk contains far more than sufficient peroxidase, up to 0.4 µM. The SCN^- content is variable (it depends on feed, as it is mainly derived from glucosides in species of *Brassica* and *Raphanus*) and mostly ranges from 0.02 to 0.25 mM. If the concentration is indeed 0.25 mM, SCN^- is, in conjunction with peroxidase, active against many bacteria that have no catalase and thus produce H_2O_2 (e.g., all lactic acid bacteria, although some of them have enzyme systems that metabolize H_2O_2, so that they are not inhibited or are less so). Fresh milk contains no H_2O_2, but if a little is added, say 0.25 mM (i.e., far too little to be active as such against bacteria or to cause oxidative defects), the system also inhibits catalase-positive organisms, like most gram-negative bacteria. If sufficient SCN^- is also present (either naturally or added), an effective preservative results, and even in a heavily contaminated milk bacterial growth may be prevented for, say, 24 h at 15°C. Addition of some glucose oxidase (EC 1.1.3.4/5) also induces formation of H_2O_2, and it may be that the natural milk enzyme xanthine oxidase (EC 1.1.3.22) can do the same under certain conditions. The natural content of catalase (EC 1.11.1.6) in milk does not interfere with the sys-

tem, even if it is high (mostly because of a high somatic cell count). Nevertheless, the inhibitory effect in milk is quite variable, largely because of the variation in thiocyanate content.

Inhibitors may enter the milk by contamination. This is undesirable, not only because fermentation may be impaired but because these substances may be detrimental to the consumers' health. Antibiotics like penicillin may be present because they have been infused into the udder to control mastitis, and they can be found in the milk up to about 3 days after infusion. In particular, some lactic acid bacteria are sensitive to these antibiotics. Disinfectants used to treat milking or processing equipment may contaminate the milk easily and then inhibit or even kill bacteria. In some countries, H_2O_2 may be added to raw milk as a preservative (10–15 mM); it is removed by addition of catalase before processing of the milk.

Treatment of milk may profoundly alter its suitability as a substrate for bacteria. The most important is heat treatment (Chapter 6), which kills bacteria and may activate sporulation but also alters the milk. Inhibitors are inactivated; this pertains to the immunoglobulins (if heated for, say, 20 s at 76°C) and to the lactoperoxidase system (e.g., 20 s at 85°C). Consequently, pasteurization may considerably stimulate growth of bacteria (which have entered the milk afterward), the higher the heating intensity the more, up to around 20 s at 85°C. Still more intense heating may lead to formation of stimulants, such as formic acid for certain lactobacilli. See further Section 6.3.3.

Homogenization appears to inactivate the agglutinins, but it usually is applied to milk that has been heated to such an extent that the agglutinins are inactivated anyway.

Fermentation by lactic acid bacteria causes the formation of lactic acid, and this is an effective inhibitor for many bacteria if it is undissociated. Its pK is about 3.95, which implies that the inhibition is stronger for a lower pH. Hardly any bacteria can grow in milk brought to a pH of <4.5 by lactic acid, but some yeasts and molds can. Bacteria also can produce other inhibiting substances, such as acetic acid, and antibiotics. Some strains of *Lactococcus lactis* ssp. *lactis* produce the powerful antibiotic nisin.

Fermentation implies a drastic change in composition. Other such changes also may be effective in inhibiting bacteria. Conditions can be made strictly anaerobic. The water activity can be lowered to such an extent that no bacteria can grow (Section 9.1), as in milk powder (by removing water) and in sweetened condensed milk (by adding sugar). The salt added to cheese has a similar effect and also increases ionic strength. Often a combination of lack of suitable nutrients, unfavorable conditions, and inhibiting agents prevents growth; this is especially true in many types of cheese (no sugar, low redox potential, not too high temperature, high salt, low pH, lactic acid).

4.2   UNDESIRABLE MICROORGANISMS

Most microorganisms are undesirable in milk because they can be pathogenic or produce enzymes that cause undesirable transformations in the milk.

Pathogenic microorganisms that enter milk can be pathogenic for humans or animals. Human pathogens are usually classified into those causing *food infection* and those causing *food poisoning*.

Food infection implies that the food, e.g., milk, acts as a carrier for the microorganism, which enters the human body through milk. So a person can become ill, often not until a day or so after drinking the milk. In food infection, fairly small numbers of microorganism may suffice to cause illness, according to the pathogen involved. Almost any pathogenic bacterium can occasionally be present in milk in very small numbers, but if it does not grow in milk, it is very unlikely to cause illness.

In food poisoning, the microorganism forms a toxin in the food (or such a toxin contaminates the food by another route). The consumer rapidly falls ill. Large numbers of the pathogenic microorganism are usually needed to cause food poisoning. The amount of toxin produced should be large enough to give symptoms. Unlike food infection, food poisoning does not imply that the pathogenic organism is still in the food. Some toxins are more heat-resistant than the toxin-producing microorganism itself, e.g., *Staphylococcus* spp.

Non-pathogenic microorganisms by themselves would not impair milk quality. It is that the organisms require nutrients, which are obtained by producing enzymes that hydrolyze lactose, protein, fat, or other substances in the milk, in order to yield compounds suitable for their growth. These conversions cause the milk to develop off-flavors and to be less suitable for processing into retail milk and milk products, e.g., because of a decreased heat stability of the milk. Furthermore, most heating processes applied in dairy processing do not destroy all microorganisms or all microbial enzymes.

Some of the spoilage and pathogenic microorganisms will now be discussed briefly. Table 4.3 gives a survey of microorganisms important for the hygiene of milk and milk products.

4.2.1   Spoilage Microorganisms

Milk is a suitable culture medium for many microorganisms, and an attempt to discuss them all would be beyond the scope of this book. Suffice it to mention some groups of bacteria, often consisting of several genera, that are responsible for a certain type of deterioration or are typical of the source of contamination or of the treatment of milk.

Lactic acid bacteria. These bacteria mainly produce lactic acid from carbohydrates such as lactose. They are widespread and include the genera *Lactococcus*

TABLE 4.3 Survey of Some Microorganisms and Groups of Organisms that Are Essential for Milk and Milk Products

Name	Source	Growth in raw milk	Heat resistance[1]	Pathogenicity	Spoilage
Bacillus anthracis	Diseased cow, soil	−	+	Anthrax	No
Bacillus cereus	Feed, dung, soil, dust	++	+	Food poisoning	Sweet curdling, ''bitty cream'' in pasteurized milk and cream
Bacillus subtilis and *B. stearothermophilus*	Feed, dung, soil, dust	++	+	Probably not	Spoil sterilized milk
Brucella abortus	Diseased cow	−	−	Contagious: abortion (cow), Malta fever? (humans)	No
Campylobacter jejuni	Dung, water	−	−	Intestinal disorder	No
Clostridium botulinum	Soil, contaminated water	−	+	Botulism	No
Clostridium perfringens	Soil, dung, contaminated water	(+)	+	Intestinal disorder (infant formulas)	No
Clostridium tyrobutyricum	Soil, silage, dung	−	+	Not	''Late blowing'' in cheese
Coliforms	Feces, milking utensils, contaminated water	++	−	Mastitis, intestinal disorder	Spoil milk and cheese
Corynebacterium bovis	Teat canal	+	−	Not	No
Corynebacterium pyogenes	Interior udder, flies	+	−(?)	Mastitis	Hardly
Coxiella burnetii	Infected cattle, dung	−	−	Q fever (humans)	No
Lactobacillus spp.	Milking utensils, milk in churns, milking parlor	++	−	Not	Sour milk

TABLE 4.3 Continued

Name	Source	Growth in raw milk	Heat resistance[1]	Pathogenicity	Spoilage
Lactococcus lactis	Milking utensils, milk in churns, milking parlor	++	–	Not	Sour milk
Leptospira (hardjo)	Infected cattle, urine, contaminated water, surface water		–	Leptospirosis	No
Listeria monocytogenes	Soil, feed, dung	+	–	Meningitis	No
Microbacterium lacticum	Milking utensils	+	+	Not	Grow in pasteurized products
Micrococcus spp. (1)	Teat canal, skin, milking parlor	+	–	Probably not	Hardly
Micrococcus spp. (2)	Milking utensils	+	+	Not	Grow in pasteurized products
Molds	Dust, dirty surfaces, feed	+/–	–	Some produce toxins	Spoil cheese, butter, sweetened condensed milk
Mycobacterium tuberculosis	Diseased cow or milker	–	–	Mastitis, tuberculosis	No
Mycobacterium paratuberculosis	Cow	–	–	Weak	No

Organism	Source				Hydrolyze protein and fat in cold-stored milk
Psychrotrophs, e.g., *Pseudomonas*	Milking utensils, cold-stored milk, polluted water	++	–	Occasionally?	Hydrolyze protein and fat in cold-stored milk
Salmonella, Shigella	Dung, polluted water	+	–	Intestinal disorder, mastitis	No
Staphylococcus aureus	Teat canal, interior udder, skin, diseased milker	++	–	Food poisoning, mastitis, ulcers	Hardly
Staphylococcus epidermidis	Teat canal, skin, milking parlor	++	–	Probably not	Hardly
Streptococcus agalactiae, S. dysgalactiae, S. pyogenes, S. uberis	Interior udder, milking parlor	++	–(?)	Mastitis	Sour milk
Streptococcus thermophilus	Milking utensils, milk in churns, milking parlor	++	+	Not	Sour milk
Vibrio cholerae	Polluted water, diseased milker	–	–(?)	Cholera	No
Viruses	Cow, humans, milking parlor	–	±	Many are, e.g., foot-and-mouth disease virus	No
Yeasts	Dust, milking utensils	+/–	–	Not	Spoil cheese, butter, sweetened condensed milk

[1] A plus sign means that a suspension of the organism concerned is not, or is not fully, killed by heating to 63°C for 30 min, which corresponds roughly to 72°C for 20 s. Variation in heat resistance may occur within one species and even one strain.

and *Lactobacillus. Lactococcus lactis* sspp. *lactis* and *cremoris* grow rapidly in milk, especially above 20°C. So milk mostly turns sour if kept uncooled. Before the milk is considered truly sour it has become unfit for processing, mainly because of the loss of heat stability. The mesophilic lactic acid bacteria are killed by low pasteurization (e.g., 15 s at 72°C) and largely even by thermalization (e.g., 15 s at 65°C). Low pasteurization does not kill thermophilic lactic acid bacteria such as *Streptococcus thermophilus*. Some streptococci are pathogenic to humans and animals (mastitis).

By contrast, the dairy manufacturer exploits lactic acid bacteria in making fermented milk products, including yogurt, cheese, and butter. Carefully selected strains of bacteria are grown under controlled conditions.

Coliforms belong to the Enterobacteriaceae and are widespread, e.g., in the digestive tract. They include *Escherichia coli* and *Aerobacter aerogenes*, but several other genera and species are involved. They grow rapidly in milk, especially above 20°C, and attack proteins and lactose, as a result of which gas is formed and the flavor of the milk becomes ''unclean.'' Some of the *E. coli* strains are pathogenic for humans.

Low pasteurization kills the coliforms to virtually the same extent as *Mycobacterium tuberculosis* (Section 4.2.2). This as well as the fact that the organisms occur widely has led to their use as indicator organisms. If coliforms are absent, the heated product has been heated sufficiently and has most likely not been recontaminated, and so pathogenic microorganisms, apart from heat-resistant ones, will most likely be absent.

Psychrotrophs, also designated pseudomonads or gram-negative rods, occur widely and include the genera *Pseudomonas, Achromobacter, Flavobacterium*, and *Alcaligenes*. Psychrotrophs grow readily at low temperatures ($<$15°C); in milk they proliferate even at a temperature as low as 4°C. Their optimum temperature is far higher, 20–30°C. The organisms produce proteases and lipases, and thus attack protein and fat, causing ''putrid'' and rancid off-flavors. Unlike the bacteria themselves, the enzymes produced can be highly resistant to heat and may cause off-flavors and alter physicochemical properties even in stored UHT milk. For example, they can hydrolyze protein, by which the milk becomes bitter and eventually more or less transparent. More than 5×10^5 psychrotrophs per ml of original milk can be harmful. In low-pasteurized and in raw milk flavor defects do not occur until numbers are over 10^7 ml^{-1} because of the brief storage time and low storage temperature of the milk.

Heat-resistant bacteria. Some bacteria, including *Microbacterium lacticum*, thermophilic streptococci, and certain *Micrococcus* species do not form spores, but the vegetative cells survive low pasteurization. Heating above, say, 80°C for 20 s kills them. The organisms are chiefly encountered in places where other bacteria die due to high prevailing temperatures, e.g., the high temperature used during cleaning of milking units. These bacteria are not very active in cold-

stored milk, but they are undesirable because they are still present in the heated product and can grow out if conditions are favorable, especially at high ambient temperatures.

Spores of bacteria. The genera *Bacillus* (aerobic or facultatively anaerobic) and *Clostridium* (strictly anaerobic) can form spores. Most of these survive fairly intense heat treatment. They originate especially from soil, dust, and dung, and also from cattle feed. Some important species and their effects are as follows:

B. cereus can spoil pasteurized milk by causing sweet curdling, a bad off-flavor, or clumps of fat globules. It is not very heat-resistant. Organisms may grow at temperatures down to about 7°C.

B. subtilis and *B. stearothermophilus* are sufficiently heat-resistant to spoil sterilized milk if insufficiently heated.

C. tyrobutyricum belongs to the butyric acid bacteria and can cause ''late blowing,'' which is a serious defect in such cheeses as Gouda or Emmentaler. Formation of gas, including H_2, leads to large holes and cracks. Production of butyric acid from the lactic acid in the cheese causes an awful flavor (Section 4.3.2.3).

The vegetative cells of spore-forming bacteria and the spores of yeasts and molds generally are not heat-resistant.

4.2.2 Pathogenic Microorganisms

Some pathogens important for milk and milk products will be discussed (see also Section 23.8). In many countries the situation regarding these organisms is fairly satisfactory. Occasionally, however, food infections occur caused by consumption of raw milk. In tropical and subtropical countries, the risk may be greater. Generally, drinking of raw milk is inadvisable.

Mycobacterium tuberculosis can originate from the cow and the milker. The bovine type of *M. tuberculosis* is also pathogenic to humans. Among the non-spore-forming pathogenic organisms, *M. tuberculosis* is the most heat-resistant; it is killed by low pasteurization of the milk, e.g., 15 s at 72°C. Incidentally, beverage milk should be pasteurized to inactivate alkaline phosphatase to the extent that it is no longer detectable (Section 14.1.1). This is mainly because inactivation of this enzyme ensures that *M. tuberculosis* has been killed. The related *M. paratuberculosis* may also occur in milk, but it is very unlikely to be pathogenic to humans.

Staphylococcus aureus often occurs in the udder of a cow with mastitis. The bacterium is also abundant in humans. Some strains can form a heat-resistant toxin and cause inflammation (ulcers). Large numbers are required to form the toxin. Growth can be slowed down by low temperature (milk), low pH, and antagonistic components formed by lactic acid bacteria (e.g., in cheese). Low pasteur-

ization kills *S. aureus*. All of these factors limit the frequency of food poisoning by *S. aureus* through milk and milk products, despite the fairly general presence of the organism in raw milk.

Salmonella and *Shigella* spp. occur widely in nature, e.g., in dung and polluted water. They can cause intestinal disorders. Low pasteurization is adequate to kill them. Milk and milk products are hardly ever responsible for food poisoning by these bacteria.

Campylobacter jejuni belongs to the family Spirillaceae and can occur in the intestinal tract of many animals. *C. jejuni* is often responsible for enteritis. Diarrhea and abdominal cramps are the main features of the disease. Milk is usually contaminated by dung, possibly also through mastitis. The organism can continue growing for a few days in raw milk at low temperature, but it is very heat-sensitive and will not survive low pasteurization. It dies rapidly in cheese, partly because of the low pH. So far, a few outbreaks have been reported as due to raw milk.

Listeria monocytogenes is often found in nature. It is pathogenic to humans and animals, and a severe infection can even be fatal. Some cases of contamination through milk are known. The organism is aerobic and can grow at a temperature as low as 5°C; it is killed by usual pasteurization.

Vibrio cholerae can occur in polluted water. The milker, if suffering from cholera, can contaminate the milk.

Coxiella burnetii belongs to the family Rickettsiae and causes Q fever in humans. It can occur in cows, goats, and sheep, and may be carried by ticks. The organism can cause mastitis but animals are often carriers without becoming ill. Low pasteurization, i.e., 15 s at 72°C, kills the bacteria but 30 min at 60°C does not. If holder pasteurization is applied, the heating temperature should be adjusted to a few degrees over that required for inactivation of alkaline phosphatase.

Bacillus cereus spores occur everywhere, e.g., in soil, dust, and feed. They survive pasteurization and, accordingly, are usually present in pasteurized milk. Psychrotrophic strains are occasionally found (some growth at 7°C). *B. cereus* can produce a toxin. However, large numbers are needed that obviously spoil the milk (awful flavor, sweet curdling), so that it will be neither consumed nor processed. Because of this, the health hazard is small. Some food products containing much starch pose a greater risk because spoilage is far more difficult to detect. If milk spoiled by *B. cereus* is used for preparation of foods, it may possibly cause food poisoning (intoxication). Heating to a temperature above 100°C kills the spores of *B. cereus*. Among the *Bacillus* spp., *B. cereus* belongs to the least heat-resistant.

Clostridium botulinum is a dreaded spore-forming bacterium, sometimes occurring in soil and surface water. It causes botulism, which results from an exceedingly poisonous toxin, formed during growth in food products. Milk and

dairy products are never the cause of botulism, though *C. botulinum* can occur in milk. Milk is too aerobic to allow growth of the microorganism. Most cheese is anaerobic and has a low redox potential, but contains no carbon source suitable for the organism. Industrial sterilization as used for milk products such as sterilized milk or evaporated milk kills any *C. botulinum* present.

Clostridium perfringens (= *C. welchii*). Spores occur in soil, dung, and consequently are often present in raw milk. However, milk and milk products are hardly ever the cause of food poisoning by this organism, though it produces a toxin during sporulation in the digestive tract. This is because *C. perfringens* is outnumbered by other bacteria in raw milk, and large numbers of vegetative cells of the bacterium are required to cause illness. Most cheese does not contain a suitable carbon source. Sterilization as applied in the dairy factory kills *C. perfringens*. Babies are more susceptible to *C. perfringens* than adults. Because of this, milk intended for manufacture of baby formulas must be heated sufficiently, i.e., to about 100°C or higher, to kill the spores of *C. perfringens*.

4.3   SOURCES OF CONTAMINATION

4.3.1   Microorganisms Present in the Udder

A distinction should be made between healthy and unhealthy cows, although it may be vague, especially for some types of mastitis.

4.3.1.1   Healthy Cows

In most cows, no microorganisms are present in the milk in the alveolus, duct, cistern, and teat cistern, but they are in the teat canal and the sphincter of the teat, mainly non-heat-resistant *Micrococcus* and *Staphylococcus* spp. and *Corynebacterium bovis*. Sometimes other bacteria are also involved. During milking, these bacteria enter the milk. Directly after milking, their number varies widely among cows, from hardly any to about 15 000 ml^{-1}; the colony count of aseptically drawn milk of healthy cows is usually low, e.g., <100 ml^{-1}. At 5°C the bacteria hardly grow and after low pasteurization these organisms can often not be detected. Obviously, microbially high-grade milk can be collected from healthy cows.

The cow has several defense mechanisms to keep microorganisms away from the udder.

- The sphincter of the teat
- Bacteriostatic and bactericidal agents present in the keratin material of the teat canal and in the milk itself, and the leukocytes in the milk
- The ''rinsing effect'' due to discharge of the milk

4.3.1.2 Unhealthy Cows

When a cow is ill due to microbial infection, the organisms involved can enter the milk. In the case of mastitis, pathogenic organisms are already present in the udder and thereby in the milk. Because of this, mastitic milk usually has a high count. Some of these mastitic organisms, including *Mycobacterium tuberculosis*, certain streptococci, *Staphylococcus aureus*, and certain strains of *Escherichia coli* are also pathogenic to humans.

If organs other than the udder are inflamed, pathogens may directly enter the milk through the body, especially if the cow is also mastitic. Naturally, the organisms can also enter the milk through, for instance, dung or urine. Among such organisms that are pathogenic to humans are *Leptospira* serotype hardjo, *Mycobacterium tuberculosis, Campylobacter jejuni, Listeria monocytogenes, Bacillus anthracis* (causes anthrax), *Brucella abortus* (causes an illness resembling Malta fever in humans), and the foot-and-mouth-disease virus. Obviously, it is essential to exclude milk of diseased animals from processing and to heat the milk in order to kill any pathogens. The drinking of raw milk is highly inadvisable.

4.3.2 Contamination During and After Milking

The hygienic measures taken during and after (mechanical) milking essentially determine what foreign microorganisms enter the milk, including human pathogens. This applies also to their numbers. The count of properly drawn mixed milk from healthy cows is about 10 000 ml^{-1}, sometimes even less. If, however, the hygienic standards during milking are poor, freshly drawn mixed milk can have a much higher count, up to one million ml^{-1}. Potential sources of contamination of milk together with the characteristic microorganisms involved will now be discussed.

4.3.2.1 The Cow

During milking, microorganisms can enter the milk from the skin of the teats, which often are contaminated by dung, soil, or dust. Flakes of skin, hairs, and dirt from the feet and flanks can also enter the milk. Several types of microorganisms can contaminate the milk, including coliforms, fecal streptococci, other intestinal bacteria, bacterial spores (mostly *Clostridium* spp.), yeasts, and molds. Some of these microorganisms are human pathogens.

Appropriate housing and care of the cows is an essential measure to promote clean udders. As a result, dry treatment, including removal of loose dirt, suffices at milking. Such a dry treatment, moreover, causes less leakage of milk against the teats. Fewer bacteria then become detached from the teat skin. Dirty udders have to be cleaned thoroughly before milking. However, the complete removal of bacteria is impossible.

4.3.2.2 Soil, Dung, Dust

All of these contaminants can reach the milk and thereby increase counts. Moreover, spores of bacteria, yeasts, and molds also occur in air. Well-known is *B. subtilis*, originating from hay dust. The spores can enter milk through air sucked in during mechanical milking, or fall directly into the milk during milking in open milking pails. The cleanliness of the milking parlor and the restfulness of the cows during milking are among the factors determining contamination of the milk.

4.3.2.3 The Feed

Feed often contains large numbers of microorganisms. Feed can sometimes fall directly into the milk but, more significantly, certain microorganisms in the feed survive passage through the digestive tract and subsequently enter milk through dung; it includes some human pathogens. Spore-forming bacteria, including *Bacillus cereus*, *B. subtilis*, and *Clostridium tyrobutyricum*, which can spoil milk and milk products, are especially involved. Large numbers of *C. tyrobutyricum* occur in silage of inferior quality. The bacterial spores survive low pasteurization of cheese milk, to which a more intense heat treatment cannot be applied, and may cause ''late blowing'' in some types of cheese (Section 24.2). Accordingly, high-quality silage is paramount and contamination of the milk, e.g., by dung, should be rigorously combatted. In some regions, the use of silage is strictly prohibited, e.g., in Switzerland in areas where Emmentaler, and in northern Italy where Parmesan cheese is made. Incidentally, when the cows suffer from diarrhea (caused, for instance, by feeding much concentrate), this increases the contamination of the milk by dung.

4.3.2.4 Milking Unit

Contact infection poses the largest threat of contamination to almost all foods, including milk. Poorly cleaned and disinfected milking equipment can contain large numbers of microorganisms. Since these organisms generally originate from milk, they will grow rapidly and can decrease quality. Residual milk often contains about 10^9 bacteria ml^{-1}, and even 1 ml of such milk entering 100 L of milk during the next milking would increase the count by 10 000 ml^{-1}.

The methods of cleaning and disinfection applied largely determine the species of the contaminating organisms. If high temperatures are used and cleaning and disinfection of milking utensils are unsatisfactory, the main species will be heat-resistant, including micrococci, *Microbacterium lacticum*, some streptococci and spore-forming bacteria. If, on the other hand, low temperatures are used, lactic acid bacteria, e.g., *Lactococcus lactis*, pseudomonads, and coliforms will mainly be involved. Use of milking equipment that can be adequately cleaned and disinfected is thus paramount. Small cracks in worn-out rubber units and ''dead ends'' in the equipment that are insufficiently rinsed should be avoided.

TABLE 4.4   Contribution of Some Sources of Contamination on the Colony Count of Raw Milk[1]

Source of contamination	Estimate of the contribution to the count (ml^{-1})
Udder of a healthy cow	Up to several thousand
Udder of a mastitic cow	Up to several million
Skin of cow	A hundred up to several thousand
Milking parlor (soil, dung, dust, air)	Up to a thousand
Feed	Up to a thousand
Milking unit	A thousand up to several million
Water for cleaning, rinsing	Up to several thousand
Good milker	Little

[1] Approximate examples.

4.3.2.5   Water Used

Tap water may be of good quality. Any private water supply must be examined at intervals. Surface water can contain many microorganisms, including human pathogens, and it must therefore on no account be used for cleaning and rinsing. Gram-negative rods like *Pseudomonas, Achromobacter, Flavobacterium*, and *Alcaligenes* spp., most of which are psychrotrophic, often occur in contaminated water (also in dung, soil, and poorly cleaned utensils). Especially in tropical countries, the water may have very high counts.

4.3.2.6   The Milker

The milker affects many of the factors mentioned and thereby the microbiological quality of the milk. He can also contaminate the milk directly, e.g., from his hands. If the milker suffers from a microbial infection, he might directly contaminate the milk with pathogens.

Table 4.4 gives an outline of the contribution of some sources of contamination to the count of milk.

4.4   HYGIENIC MEASURES

In discussing measures that would result in a satisfactory bacteriological milk quality, contamination by undesirable bacteria should be distinguished from growth of the bacteria in milk. Butyric acid bacteria, for example, cannot grow in milk, but the presence of more than 1 spore/ml of milk is undesirable in the production of some types of cheese. Psychrotrophic bacteria, however, grow rapidly in milk, and contamination by 10^2–10^3 ml^{-1} during milking is hard to avoid.

Counts lower than 10^5 ml^{-1} do no harm. The distinction should also be made in relation to food infection and food poisoning. Hygienic measures should aim at suppressing pathogens and inhibiting spoilage organisms. These two subjects will now be discussed.

4.4.1 Protection of the Consumer Against Pathogenic Microorganisms

The following are the main reasons why contamination of raw milk by pathogens and growth of these organisms in milk during storage should be avoided as much as possible.

    a. During microbial growth in raw milk, toxins may be formed. Some toxins are fairly heat-resistant.
    b. Some pathogens survive heat treatments such as pasteurization. Fortunately, this is exceptional. The higher the count in the raw milk, the more organisms may survive heat treatment. This is of importance if the heat treatment applied leaves only a small margin.
    c. The heavier the contamination of raw milk by pathogens, the greater the risk of recontamination of the heated milk.

Contamination of raw milk by pathogens can never be ruled out. Milk intended for liquid consumption or for transformation into milk products is therefore often required by law to be heated to such an extent that the common pathogens are killed; this implies at least low pasteurization.

    The drinking of raw milk is highly inadvisable. However, (semi)hard cheese made from raw milk is harmless for the consumer. The lactic acid bacteria rapidly hydrolyze the lactose to yield lactic acid, which is not a suitable carbon source for most pathogens. As a result, pH decreases rapidly to below, say, 5.5, which is unfavorable for many pathogens. The redox potential drops to a low value, about -150 mV, which prevents aerobic microorganisms from growing. Moreover, the lactic acid bacteria form compounds that are antagonistic to some pathogens. Most pathogens, if present, die within a few weeks (see Table 23.6). However, there is a real danger that pathogens are present in soft cheeses made from raw milk.

    Measures taken to prevent growth of spoilage organisms also stop growth of pathogenic bacteria that can produce heat-resistant toxins. Pasteurized milk is therefore among the safest food products of animal origin.

4.4.2 Measures Against Spoilage Organisms

A low contamination by microorganisms is the first aim. To achieve this, the sources of contamination should be known. Some are found before milking, espe-

cially in housing (clean cows) and fodder production (butyric acid bacteria). Cleaning and disinfection of the milking equipment is essential (Chapter 12). It is specifically meant to remove and kill bacteria. Bacteria originating from inadequately cleaned equipment usually have no lag phase and can grow rapidly in milk (Fig. 4.2).

Cooling is the main means of slowing down the growth of bacteria in milk. The maximum storage time of milk closely depends on storage temperature. A satisfactory operation of refrigerated milk tanks on the farm is essential. However, cooling of milk kills no bacteria and it cannot remedy unsatisfactory hygiene.

In dairy factories, the raw milk received often is not simply stored before processing, but is thermalized and then cooled to below 4°C. Thermalization is a mild heat treatment, e.g., 15 s at 65°C. It kills nearly all psychrotrophic bacteria, which are not at all heat-resistant. In this way, growth of these bacteria to harmful numbers during cold storage of the milk in the factory is prevented, as is the formation of heat-resistant enzymes (lipases and proteinases). Thermalization kills part of the other bacteria, including many lactic acid bacteria.

SUGGESTED LITERATURE

- An advanced and comprehensive textbook on food microbiology, including aspects of milk, is:

     D. A. A. Mossel, J. E. L. Corry, and C. B. Struijk. *Essentials of the Microbiology of Foods*, Wiley, Chichester, 1995.
- Microbiology of milk (and of some milk products) is treated in:

     R. K. Robinson, ed. *Dairy Microbiology*, Vol. 1, *Microbiology of Milk*, and Vol. 2, *Microbiology of milk products*, 2nd ed., Elsevier, London, 1990.
- Microbiology of raw milk is discussed in an IDF report:

     Factors influencing the bacteriological quality of raw milk, International Dairy Federation, Document 120, Brussels, 1980.
- Methods for quality determination of raw milk are given in:

     Bulletin of the International Dairy Federation No. 256, Brussels, 1990.

II

PROCESSES

5

General Aspects

Before coming to specific processes, we will discuss some general considerations about milk processing and quality assurance.

5.1 INTRODUCTION

Milk is the raw material for the manufacture of several food products. These products are predominantly made in dairy factories (or dairies, for short). Their mode of operation is dominated by the properties of the raw material. Some typical *characteristics* of the dairy industry are as follows:

  a. Milk is liquid and homogeneous (or it can readily be made homogeneous). This implies that transport and storage are relatively simple and greatly facilitates the application of continuous processes.
  b. Milk properties vary according to source, season, and storage conditions, and during keeping. This may imply that processes have to be adapted to the variation in properties.
  c. Milk is highly perishable and the same holds true for many intermediates between raw milk and final product. This requires strict control of hygiene and storage conditions.
  d. Raw milk may contain pathogenic bacteria and some of these can thrive in milk. This also requires strict control of hygiene and stabilization processes.
  e. Raw milk generally is delivered to the dairy throughout the year, but in varying quantities (in some regions there is even no delivery during part of the year). Because the milk must be processed within at most

a few days, this implies that the processing capacity of a dairy can generally not be fully used during most of the year.

f. Milk contains several components and it can be separated in fractions in various ways, e.g., in cream and skim milk, in powder and water, or in curd and whey. Moreover, several physical transformations and fermentations can be applied. This means that a wide variety of products can be made.

g. Besides milk, fairly small amounts of raw material are needed for the manufacture of most milk products, but consumption of water and energy may be high.

h. One and the same unit operation often can be applied in the manufacture of a range of products. This includes heat treatment, cooling, cream separation, and homogenization.

Nearly all process steps, or *unit operations*, that are applied in food processing are applied in the dairy. They can be grouped as follows:

a. *Transfer of momentum*: pumping, flow.
b. *Heat transfer*: heating and cooling.
c. *Mixing/comminution*: stirring, atomization, homogenization, recombination. The last two of these can also be considered physical transformations.
d. *Phase separation*: skimming, separating milk powder from drying air, part of the churning process.
e. *Molecular separation*: evaporation, drying, membrane processes, crystallization (of water, lactose, milk fat).
f. *Physical transformation*: gel formation (as due to renneting or acidification of milk), important elements of butter making, making of ice cream, etc.
g. *Microbial and enzymic transformation*: production of fermented products, cheese ripening.
h. *Stabilization*: pasteurize, sterilize, cool, freeze. At least one of these operations is virtually always applied. Most stabilization processes are also, or even primarily, aimed at ensuring food safety.

In some cases, general knowledge of food process engineering may suffice to apply these unit operations. Some operations that are essential in dairy manufacturing are, however, not treated or are hardly treated in texts on food engineering. Moreover, the process affects the material, which is why it is applied, but the material also affects the process, of which numerous examples are to be found in the ensuing chapters. Often, such mutual interactions are intricate, specific, and of practical importance. Consequently, a thorough knowledge of the physics,

chemistry, and microbiology of milk and its components is needed to understand the changes, both intended and undesired, occurring in the material during processing.

   Objectives. In the development of processes for the manufacture of food products, several constraints have to be taken into account. These include availability of skilled staff, materials, machinery, and specific knowledge, as well as legal conditions. However, the objectives of the production process are paramount. The ensuing requirements can be grouped as follows:

   a. *Safety of the product for the consumer*. The health of the consumer can be threatened by pathogenic bacteria (or their toxins) and by toxic or carcinogenic substances. The first of these provides nearly always by far the most serious hazard. These aspects are generally discussed in Section 5.2, and more specifically in Chapter 4 and the product chapters.
   b. *Quality of the product*. Apart from product safety, which may be considered a quality aspect, this generally involves:
      • Nutritional value.
      • Eating quality: taste, odor, mouthfeel.
      • Appearance: color, texture.
      • Keeping quality or shelf life, i.e., the time a product can be kept before it significantly decreases in quality.
      • Usage properties, e.g., spreadability of butter, whippability of cream, dispersibility of milk powder; and, in general, ease of handling.
      • Emotional values: a wide range of aspects, greatly varying among consumers. To be sure, most of the aspects just mentioned may also be subject to ''emotional'' considerations by the consumer.
      The quality requirements may vary widely among products, and even if they are the same (e.g., the shelf life) it may demand different measures to meet the requirements.
   c. *Quality of the process*. The process should be safe and convenient for the staff involved as well as for other people around. It should not cause environmental problems, such as pollution or excessive use of exhaustible resources (e.g., energy and water).
   d. *Expenses*. Often, the necessity to maintain the processing costs within limits is overriding. It may concern the price of raw materials (including packing), the use of energy, the equipment expenditure, the labor intensity, etc. Also the flexibility and the complexity of the process, with the ensuing probability of making mistakes (poor quality or even discarding of products), may affect production costs. The same holds true for the costs of storage.

It may be clear that the objectives are manifold and often mutually conflicting. This means that process optimization may be far from easy to achieve.

5.2 QUALITY ASSURANCE

Quality assurance is paramount in all food manufacture and handling. It involves a coherent system of activities that assures (guarantees) that the products made meet a set of defined quality marks. Specific aspects will be discussed at various places throughout the rest of this book. In this section some general considerations are given.

5.2.1 Concepts

Quality can be defined in various ways. A well-known definition (by J. M. Juran) is: ''Quality is fitness for use.'' This needs some elaboration. A product or a service is fit for use if it meets the expectations of the user. However, it is far from easy to establish what these expectations are. This is because the expectations vary among consumers, often widely so, and generally depend on conditions under which a product is purchased or used. Moreover, several quality marks are highly subjective and it is difficult to translate these into measurable product attributes. A high quality does not merely mean that the product complies with legal requirements or preconceived ideas of the manufacturer. Marketing specialists and technologists should cooperate in establishing the desired quality marks.

Food technologists then play a key role in translating quality aspects into defined criteria and in developing methods for determining whether and to what extent a criterion is met. In Section 5.1, a list of quality aspects is given. For some of these, the value can be estimated by more or less objective methods (e.g., safety, shelf life, dispersibility), and others can only be assessed by consumer panels (e.g., flavor). It is often tried to establish correlations between objective criteria and consumer opinions, e.g., between acidity or diacetyl content of fermented milks and their flavor, or between a rheological parameter and the subjectively observed spreadability of butter.

To assure that a high quality is obtained, it does not suffice to define criteria and then to inspect whether those are met. Quality must be controlled (enforced) and is thus a management function. The current approach is a system of integrated or *total quality management*. It involves integration at three levels:

a. Throughout the production chain, i.e., from the farm to the consumer. It may even have to start before the farm, for instance in the design of milking machines or in the specifications for concentrates fed to the cows. Distribution of the products made also involves several steps that bear on product quality.

b. For the product in the widest sense, including service. This would in-

       volve the way in which the product reaches the consumer and the infor-
       mation given about the product.
    c. Throughout the organization, i.e., at all hierarchical levels and in all
       departments.

The quality concept has to be built in from the beginning: in the definition of
the product and its positioning in the market; in the development of the process
to make it; in the design of the equipment (e.g., ''cleanability''); in the specifica-
tions for the raw materials; in the planning of the logistics for distributing the
product; and so on. In other words, quality begins with the *design*: can good
products be made by the planned procedures? The next question is whether the
desired quality can be *reproduced*: does every item produced comply with the
set quality criteria? For the latter, a control system has to be installed. However,
the general rule should be that prevention is better than cure.

      Of greatest concern is the *safety* of the product for the consumer. Milk may
contain several types of pathogenic bacteria. Because their presence is largely
determined by chance, and because a single bacterial cell can in principle be
dangerous (since some pathogens can grow in milk), safety cannot be assured
by selecting and inspecting samples. In practice, it is almost never possible to
check every unit of the product. Consequently, other measures must be taken,
such as

    a. Treating the raw milk in such a way that all of the pathogenic bacteria
       that can be present and harmful are killed.
    b. Prevention of recontamination of intermediates and end product. This
       requires strictly enforced hygienic measures and packing.
    c. Transformation of the material into a product in which pathogens can-
       not grow; a good example is fermented milk. Preferably, any pathogens
       present will die off.

A combination of the three treatments will give the least chance for ''accidents''
to happen. However, all of these measures, especially the third one, cannot always
be taken. Consequently, a rigorous inspection and control system must be estab-
lished.

      Health hazards due to toxic or carcinogenic concentrations of substances
in the product are very rare in milk products. Most contaminations with hazardous
substances are restricted to one farm, and the dilution achieved by mixing the
milk with that from several other farms generally causes the concentration in the
final product to be far below the toxic level. Sampling of the final product will
generally establish the contamination, and sampling of all road tankers, and sub-
sequently of individual deliveries, will then readily locate the source of contami-
nation.

5.2.2 HACCP

HACCP stands for hazard analysis/critical control points. It is a method to establish for an existing production process what control measures are essential to assure the safety of the products made. The same method can be applied for other quality characteristics, but the emphasis generally is on safety. HACCP should be applied separately to every manufacturing process actually in operation; this means a separate system for every product or group of closely related products. The main features of the method are what the name says: make an analysis of the potential hazards, identify critical points in the process, and establish criteria for control.

HACCP is also a control system applied after the analysis has been made. It involves corrective measures where needed, e.g., via feedback or control loops that adjust process variables if needed; a simple example is adjustment of a heating temperature. An HACCP study may reveal that the process should be changed to allow efficient control.

Instruction manuals give more details, as in Table 5.1. The product and its use are described in detail. The manufacturing process is carefully described in a flow diagram, including the control points for adjusting the process (e.g., temperature, flow rate, mixing intensity, rate at which a component is added). Each

TABLE 5.1 Procedure for Developing a Control System for the Manufacture of a Food Product, According to the European Hygienic Design Group

Stage	Action
1	Define terms of reference
2	Select the HACCP team
3	Describe the product
4	Identify intended use of product
5	Construct a flow diagram
6	On-site verification of flow diagram
7	List all hazards with each process step and list all measures which will control the hazards
8	Apply HACCP decision tree to each process step in order to identify CCPs
9	Establish target level(s) and tolerance for each CCP
10	Establish a monitoring system for each CCP
11	Establish a corrective action plan
12	Establish record keeping and documentation
13	Verification
14	Review the HACCP plan

CCP, critical control point; HACCP, hazard analysis/critical control points.

step in the process is analyzed for its potential hazards, and these are evaluated and quantified; it then is analyzed what measures can be taken to minimize the hazard, and it is finally decided, on the basis of systematic criteria, whether this is made a critical control point. If so, a monitoring scheme is devised, an essential point of which is the monitoring frequency. Monitoring too often is unnecessarily expensive and tends to demotivate the operators, monitoring too seldom may lead to an unacceptable hazard. This procedure is applied to every process step, leading to a complete HACCP system. Furthermore, a corrective action plan should be developed, i.e., what measures should be taken when some critical parameter is observed to be outside the limits set. The system should regularly be evaluated and verified during its application, and modified where needed.

An essential aspect is that HACCP systems cannot be copied. Every manufacturer has his own particulars in the process applied and in outside conditions and constraints. Moreover, the development and repeated evaluation of the system by the people involved in its application is a prerequisite for its success. Consequently, this book will not give prescriptions for HACCP systems for particular products, although potential hazards and critical points in a process will often be indicated.

5.2.3   Quality Assurance of Raw Milk

As mentioned, total quality assurance involves the full chain from the production of raw milk to the consumption of dairy products. Obtaining high-quality raw milk is a matter of particular and lasting concern for a dairy. This is because in milk production so many steps and aspects play a role and because so many individual producers are involved. Figure 5.1 illustrates various relationships between a dairy and the outside world. It follows that extensive measures for quality assurance also have to be taken in the distribution of dairy products.

Raw milk quality has several aspects, the most important being gross composition and hygienic quality. The former can readily be assessed by determination of, say, fat and protein contents in random samples; the price of the raw milk is determined by its composition. Assurance of the hygienic quality poses more problems, mainly because mistakes leading to poor quality can readily be made and because sampling and analysis of every lot delivered would often be too expensive. The actual measures to be taken will vary greatly with local conditions, problems, and regulations, but in any case milk must be sampled and analyzed on a regular basis. The success of a quality assurance system would further depend on a number of conditions:

a.   The farmer should be knowledgeable about hazards and remedies, and should be committed to deliver high-quality milk. This means that training and information should be provided.

b.   The farmer should be financially rewarded for producing milk of good
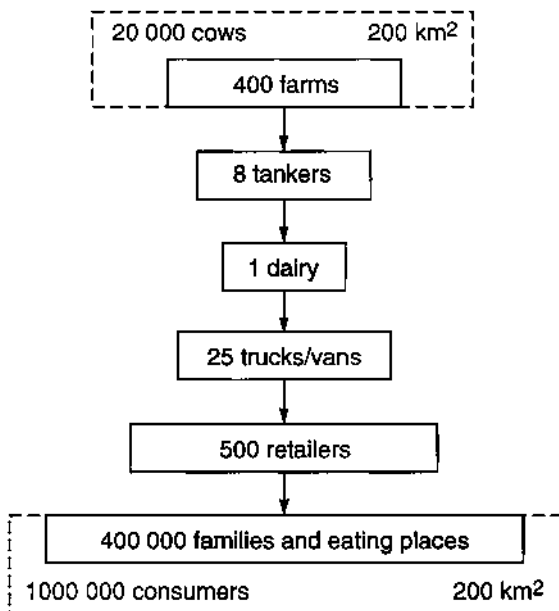
FIGURE 5.1   Relations between a dairy and its producers of raw milk and its consumers of products. Total quantity of milk processed would be 10^8 kg per annum. Hypothetical and simplified. (Courtesy of M.G. van den Berg.)

        hygienic quality, and should be penalized for delivering milk that is adulterated or potentially harmful.

c. If the farmer encounters difficulties in producing high-quality milk, help should be provided in establishing the cause and in finding remedies.

d. If the farmer suspects that his milk accidentally is of poor quality, e.g., because the cooling system has failed, he should have the option of reporting this to the dairy. The milk can then be collected separately, and any financial penalty for poor-quality milk should be restricted to the quantity of that lot.

Another problem is that raw materials for concentrate cattle feed are sometimes contaminated with substances that may reach the milk, e.g., aflatoxins. The best solution seems to be that the dairy industry and the cattle feed industry come to agreement on how to curtail such problems.

        It goes without saying that milk of unacceptable hygienic quality should always be rejected. On the other hand, quality criteria for raw milk should not be more stringent than necessary to make safe and good-quality milk products.

5.3 MILK STORAGE AND TRANSPORT

Milk storage and transport operations are aimed at having good-quality milk available where and when needed for processing. The milk should not be contaminated by microorganisms, chemicals, water, or any other substance. Obviously, the costs involved in storage and transport should be kept low, which implies that, for example, loss of milk should be minimized. Simple and effective cleaning of all the equipment involved should be possible. Furthermore, a satisfactory record of actual losses is desirable; most manufacturers determine a mass and fat balance on a daily basis.

Transport and storage refer to raw milk as well as to intermediate products.

5.3.1 Milk Collection and Reception

Milk may be supplied to the dairy in milk cans (churns) or by a tanker after it has been cold-stored at the farm (tank milk).

During transport, *milk in cans* usually has a temperature of >10°C, up to 20–30°C according to the climate. Consequently, bacterial growth can occur between milking and milk arrival at the dairy, as this interval may take as long as a day. The extent of bacterial growth depends primarily on the quality of hygiene during milking, the temperature, and the storage period (see Chapter 4). Spoilage of the milk is mainly by mesophilic bacteria and usually involves a lactic acid fermentation; however, heavy contamination with polluted water (mainly pseudomonads) may cause a nonsouring spoilage. On reception at the dairy plant, milk is cooled to <6°C, which helps to more or less stabilize its bacteriological quality for at most 2 days.

Tank milk has been kept at low temperature but for a longer time. It mainly contains psychrotrophs and consequently requires another treatment than milk in cans. Among the advantages of tank milk over milk in cans are the cheaper transport costs (if the collection routes are not too long) and a regular supply of good-quality milk, provided that the temperature of the milk at the farm and during transport is satisfactorily controlled.

On reception, the quantity of milk is recorded first. At the dairy, milk in cans is weighed by a platform balance. The quantity of tank milk is determined by metering the intake line of the milk tanker. Milk volume is then converted to weight.

Collected milk ought to be routinely examined to identify poor-quality milk supplies. A simple, rapid examination of the sensory properties would include odor, appearance, and temperature. In addition, the intake pipe of the milk tanker can be equipped with a continuously recording thermometer and a pH meter that may switch off the intake pump if the values recorded exceed a predetermined level. Incidentally, an off-flavor is more easily detected in the warmer milk in cans than in tank milk, and souring of milk can be detected easier than the growth of psychrotrophs. In addition to this simple inspection, the milk can be tested

for the presence of antibiotics, as well as its freezing point depression, acidity, and bacterial count.

It is advisable that the reception of milk in cans at the dairy occurs as soon as possible after milking. This implies twice-a-day milk collection. Often this is not practical and the evening milking is cooled by mains or well water. Once-a-day collection may, however, seriously impair the milk quality in cans. Tank milk should be refrigerated to <4°C. After 4 or 5 days storage, substantial growth of psychrotrophs may have occurred (Section 5.3.2). Consequently, tank milk can normally be kept on the farm for 3 days, i.e., six milkings, and stored for another day at the dairy before processing.

Milk can be contaminated during transport if the tanker had been inadequately cleaned. Milk tankers can contaminate milk with high numbers of psychrotrophs. This means that rigorous cleaning of the tanker and routine monitoring are essential. Furthermore, the temperature of the milk during transport must be kept low, i.e., <5°C.

Paying strict attention to the measures mentioned above will ensure a satisfactory quality of the raw material supplied. A small quantity of milk of somewhat inferior quality will have little effect due to its dilution in the large storage tanks of the dairy. However, milk supplies of poor quality should preferentially be eliminated.

5.3.2   Milk Storage

Variations in composition, properties, and quality of the raw milk directly affect the manufacturing processes as well as the composition and quality of the final products, and are therefore undesirable. Some variation is inevitable, but mixing many deliveries in large storage tanks, containing, for example, 300 000 kg of milk, results in only a small variation among lots of milk within 1 or 2 days.

5.3.2.1   Bacterial Growth

The maximum keeping quality of raw milk in storage tanks is mainly determined by the growth of psychrotrophs. Prior to processing, bacterial numbers greater than 5×10^5 ml^{-1} in milk imply a risk that psychrotrophs have produced heat-stable enzymes, i.e., bacterial lipases and proteinases, which may impair the quality of the final product. It is important to note that a high count originating from mixing a small quantity of milk containing many psychrotrophs with a large quantity of milk of a low count is more harmful than a similar count resulting from limited growth in the whole lot. This is because extracellular enzymes are predominantly produced at the end of the exponential growth phase. Examples of the growth of psychrotrophic and other bacteria in milk during storage on the farm and at the dairy are given in Figure 5.2. Initially, during storage on the farm, the total count remains almost constant and only starts to increase after 4
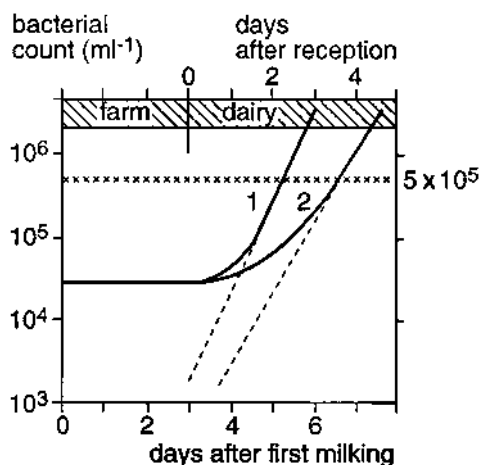
FIGURE 5.2 The growth of psychrotrophic and other contaminating bacteria of different generation times (*g*) in tank milk at 4°C. Calculated examples. Total count (—), number of psychrotrophs (---), desirable upper limit for processing (xxx). 1. Fast-growing contaminating bacteria, *g* = 6 h; 2. Contaminating bacteria of *g* = 8 h.

or 5 days. The delay in the growth of psychrotrophs to high numbers is often thought to be due to an extended lag phase at low temperatures. However, a very low initial contamination with fairly fast-growing bacteria, i.e., <10 ml^{-1}, e.g., from an improperly disinfected bulk tank, may also be responsible for the delay, as illustrated in Figure 5.2.

Depending on the age of the milk supplied to the dairy, it can be stored for another 1 or 2 days without further treatment. All milk supplies should, however, be cooled to <4°C because the temperature of milk may have increased during transport from the farm to the dairy and the generation time of bacteria is markedly shorter at high temperatures (see Table 4.1). Often, the dairy is unable to process all milk supplies within 4 days of milking. Consequently, measures must be taken to keep the raw milk for a longer time. Pasteurization (72°C for 15 s) is not desirable because it will be done later on, and pasteurizing twice may impair the quality of the finished products. A more moderate heat treatment (e.g., 65°C for 15 s, called thermalization) reduces the number of psychrotrophs considerably while leaving most milk enzymes and agglutinins intact (Section 6.3). After thermalization, the milk can be kept for another 3 or 4 days at 6–7°C without substantial increase in the bacterial count, provided that there is no recontamination by psychrotrophs. Milk should be thermalized as soon as possible after arrival at the dairy. Thermalization is a far better method for controlling

the quality of dairy products than merely cooling the raw milk, but it also is more expensive. Since many bacteria survive thermalization, considerable bacterial growth can occur at 30–40°C in the regeneration section of the heat exchanger (Section 6.4.4). Therefore, it may be necessary to clean it after operating for 4 to 6 h. The quality of thermalized milk may still be threatened by the presence of any psychrotrophs that are fairly heat-resistant, e.g., *Alcaligenes tolerans*.

   Usually, the quality of milk is examined after it arrives at the dairy. It is advisable to test the milk again just before processing. Standards for the milk quality before processing are given in Table 5.2.

5.3.2.2 Enzyme Activity

Lipase activity is usually the main problem in fresh milk (Section 3.1.5), although other milk enzymes, e.g., proteases and phosphatases, also cause changes. Therefore, extensive temperature fluctuations, in the range of 5°C to 30°C, and damage to fat globules (see below) should be avoided.

5.3.2.3 Chemical Changes

Exposure to light should be avoided because it results in off-flavors (Section 2.6.4). Contamination with rinsing water (which causes dilution), disinfectants (oxidation), and especially with Cu (catalyzes lipid oxidation) all should be avoided.

5.3.2.4 Physical Changes

The following are the main physical changes that can occur during storage:

   a. Raw or thermalized milk stored at low temperature creams rapidly (Section 3.1.4.2). Formation of a cream layer can be avoided by regular

TABLE 5.2 Examples of Standards for (Pooled) Milk Before Processing

Quality mark	Standard	Absolute limit	Unit
Acidity	17	≤ 18	°N[1]
Count (raw)	100; 95% < 250	<500	μl^{-1}
Count (thermalized)	50; 95% < 100	<250	μl^{-1}
Heat-resistant bacteria	5; 95% < 10	<25	μl^{-1}
Bacillus cereus	0.1; 95% < 0.2	≤ 1	ml^{-1}
Fat acidity	0.6; 95% < 0.8	≤ 0.9	mmol/100 g
Freezing point depression	520–525	>515	mK
Antibiotics	Not detectable		
Disinfectants	Not detectable		

[1] mmol/L.

stirring of the milk, e.g., stirring for 2 min every hour. This is often done by aeration; the air supplied should be sterile, for obvious reasons, and the air bubbles fairly large, since otherwise too many fat globules would adsorb onto the bubbles (see point b).

b. Damage to fat globules is mainly caused by air incorporation and by temperature fluctuations that allow some fat to melt and crystallize. These events can lead to increased lipolysis, to disruption of fat globules if the fat is liquid, and to clumping of fat globules if the fat is partly solid (10°C to 30°C).

c. At low temperatures, part of the casein, primarily β-casein, dissolves from the micelles to end up in the serum. This dissolution is a slow process and reaches equilibrium after approximately 24 h (Section 3.2.2.2). The dissolution of some casein increases the viscosity of the plasma by approximately 10% and reduces the rennetability of the milk. The reduced rennetability may be partly due to a changed calcium ion activity (Section 2.2.4.4). Temporarily heating milk to ~50°C or higher almost fully restores the original rennetability of the milk.

5.3.3 Transport of Milk in the Dairy

To move the milk about, a dairy needs an intricate system of pipelines, pumps, and valves, as well as controlling units. The system should be flexible while excluding such errors as milk running off or unintentional mixing of different products. To save on pumping costs, gravity is often used. Pumping of viscous products requires much energy and the common centrifugal pumps are unsuitable. These pumps are preferentially used for milk because they keep turning without great problems if the milk cannot be discharged. Following are some specific problems.

a. *Milk losses*. This concerns residues in pipes and equipment after processing, spillage, mixing of milk with different products or with water when valves are switched over. Ensuring a satisfactory discharge of the milk, avoiding ''dead ends'' in pipes, and minimizing the surface area wetted by milk all are obvious measures to reduce losses. Minimizing the diameter (D) of pipes can reduce the amount of mixing that occurs between milk and water; the volume of the mixing region is proportional to $D^{2.55}$. Milk diluted with water may be evaporated or mixed with skim milk powder or used as cattle feed. Proper operation reduces the cost due to milk losses to approximately 1% of the total cost of the raw material.

b. *Damage to milk*. Air incorporation may damage milk fat globules. Excessive shear rates and intense turbulence during transport may cause clumping, i.e., formation of visible lumps of fat, especially in cream.

In transporting cream it is thus advisable to avoid narrow and long pipes, as well as obstacles (e.g., sharp bends) in the pipeline system; the cream should not be transported at temperatures between 10°C and 40°C. Furthermore, the viscosity of products like yogurt and custard can be markedly reduced by high deformation rates occurring during transport (irreversible breakdown of structure).

c. *Bacterial growth*. During transport contamination of milk by bacteria can readily occur. Balance tanks are often situated before various kinds of processing equipment to ensure a constant milk flow rate. If the temperature in such a balance tank is high enough for bacterial growth, the tank tends to act as a continuous fermentor, allowing growth of bacteria in the milk to be processed. Leaving raw milk for some time in noninsulated pipelines favors bacterial growth. Obviously, all such situations should be avoided.

5.4 STANDARDIZING

Standardization of the composition of a milk product is needed because it is legally required or because the manufacturer sets a standard for his product. It mostly concerns the fat content, often also the dry matter content (or the degree of concentration), sometimes the protein content, or still another component.

From an economic point of view, continuous standardization is desirable: turbidity or density measurements can be applied for fat content, density, or refractive index for dry matter content. Measuring infrared reflection is also used, e.g., to determine the water content of milk powder. In continuous standardization, the mostly amplified measuring signal may control the position of a regulating valve, e.g., a valve in a cream line or in a steam supply pipe; in this way the desired content can be adjusted. To achieve this, the relationships between turbidity and fat content, between density and dry matter content, etc., in the original milk must be known. This is because these relationships are not always the same. The adjustment is often difficult because great fluctuations can easily occur when the adjustments are being made. Therefore, a double adjustment is often employed, based on measurement of the volume flows as well as a concentration-dependent variable.

After the standardization, performed tentatively or by means of continuous determination, the desirable content will have to be checked. This implies that it may be necessary to make an adjustment by addition of cream, skim milk, water, etc. Any bacterial or other contamination should be rigorously avoided. The added compound should have been treated (especially with respect to heating) in a way similar to that of the product itself.

Standardization is always subject to inaccuracy because the results of the methods of determination and the measuring or weighing of the components have

a certain inaccuracy. The same holds for determination by the supervising authority. Therefore, a certain margin should be left, e.g., twice the standard deviation. In some cases, for example with respect to the fat content of beverage milk, a deviation of $\pm0.05\%$ fat may be permitted, whereas the average value over a prolonged period should deviate by no more than 0.01% fat from the accepted standard value.

Standardization of products (e.g., beverage milk) with respect to protein content is generally not allowed. All the same, the nutritive value and the cost price of the milk greatly depend on the (variable) protein content. Technically, standardization is possible by applying ultrafiltration.

The standardization of cheese milk is discussed in Section 22.6.

SUGGESTED LITERATURE

- There are several text and reference books about dairy technology (processing and products), but most of these are very elementary. Some interesting aspects are discussed in:

  R. K. Robinson, ed., *Modern Dairy Technology*, Vol. 1, *Advances in Milk Processing*, and Vol. 2, *Advances in Milk Products*, 2nd ed., Elsevier, London, 1993.
- A general text on HACCP is:

  M. D. Pearson and D. A. Gorlett, *HACCP: Principles and Applications*, AVI, New York, 1992.
- See also:

  S. Leaper, ed., *HACCP: A Practical Guide*, Technical Manual 38, Cambden Food and Drink Research Association, Camden, 1992.

6

Heat Treatment

The manufacture of virtually all milk and dairy products involves heat treatment. Such treatment is mainly aimed at killing of microorganisms and inactivation of enzymes, or to achieving some other, mainly chemical, changes. The results greatly depend on the intensity of the treatment, i.e., the combination of temperature and duration of heating. It is also useful to distinguish between irreversible and reversible changes. The latter are often involved when milk is brought to a high temperature to facilitate some reaction or process, such as renneting of cheese milk, growth of starter organisms, efficiency of water evaporation or centrifugal separation, etc.

Heat treatment may also cause undesirable changes, although desirability may depend on the product involved or on its intended use. Examples are browning, development of a cooked flavor, loss of nutritional quality, inactivation of bacterial inhibitors, and impairment of rennetability. This often means that heat treatment should be carefully optimized.

After defining the objectives of heat treatment, the various chemical and physical reactions occurring at high temperature will be discussed. This is followed by the kinetics of the changes occurring. Finally, more practical aspects of heat treatment will be discussed. For the benefit of readers not well acquainted with the fundamentals of heat transfer, some aspects are briefly given in an Appendix (Section 6.5).

6.1 OBJECTIVES

The main reasons why heat treatment of milk is applied are the following:

1. *Warranting the safety of the consumer.* It specifically concerns killing of pathogens like *Mycobacterium tuberculosis, Coxiella burnetii, Staphylococcus aureus, Salmonella* species, *Listeria monocytogenes*, and *Campylobacter jejuni*. It also concerns potentially pathogenic bacteria that may unintentionally enter the milk. A fairly moderate heat treatment kills all of these organisms. Highly heat-resistant pathogens either do not occur in milk (e.g., *Bacillus anthracis*), or they become readily overgrown with other bacteria (e.g., *Clostridium perfringens*), or they cannot grow at all in milk (e.g., *Clostridium botulinum*), or they are pathogenic only at such high numbers (e.g., *Bacillus cereus*) that an approaching spoilage of the milk is detected long before these counts are reached. To be sure, some toxins (especially from staphylococci) can withstand moderate heat treatments.

2. *Increasing the keeping quality.* It primarily concerns killing of spoilage organisms and of their spores if present. Inactivation of enzymes, native in milk or excreted by microorganisms, also is essential. Chemical deterioration by autoxidation of lipids (Section 2.3.3) can be limited by intense heat treatment. Rapid creaming can be avoided by inactivating ''agglutinin'' (Section 3.1.4).

3. *Establishing specific product properties.* Examples are (a) heating the milk before its evaporation to increase the coagulation stability of the evaporated milk during its sterilization (Section 16.1.4); (b) inactivating bacterial inhibitors like immunoglobulins and the lactoperoxidase-CNS-H_2O_2 system (see also Section 6.3.3) to enhance growth of starter bacteria; (c) obtaining a satisfactory consistency of yogurt (Section 20.3.3); (d) coagulating serum proteins together with casein during acidification of milk (Chapter 18).

6.2 CHANGES CAUSED BY HEATING

6.2.1 Overview of Changes

Changes in milk caused by increase in temperature may be reversible or irreversible. Here we are interested mainly in irreversible or slowly reversible reactions; such changes scarcely occur at heat treatments of lower intensity than low pasteurization. All the same, reversible reactions must be taken into account because they determine the state at increased temperature, i.e., the conditions in the milk at which the irreversible changes take place. Reversible changes include the muta-

rotation equilibrium of lactose (Section 2.1.2.1) and ionic equilibria, including pH (see, e.g., Sections 1.2.2 and 2.2.4).

Numerous changes that occur on heating are discussed throughout this book. Here we give a brief survey of the main changes. The list is by no means complete. Moreover, several changes are interdependent, and the various changes may occur at very different heating intensities.

6.2.1.1 Chemical and Physical Changes

Chemical and physical changes caused by heat treatment are as follows:

1. Gases, including CO_2, are removed (if they can escape from the heating equipment). Loss of O_2 is important for the rate of oxidation reactions during heating and for growth rate of some bacteria afterward. The loss of gases is reversible, but uptake of air may take a long time.
2. The amount of colloidal phosphate increases and the $[Ca^{2+}]$ decreases (see Fig. 2.8). Again, the changes are reversible, though slowly ($\sim$24 h).
3. Lactose isomerizes and partly degrades to yield, for instance, lactulose and organic acids (Section 6.2.3).
4. Phosphoric esters, those of casein in particular, are hydrolyzed (Section 6.2.2.3). Phospholipids and some dissolved esters are also split. Consequently, the amount of inorganic phosphate increases.
5. The pH of the milk decreases, and the titratable acidity increases, mainly due to changes 2, 3, and 4 (see Fig. 6.1). All of these changes depend somewhat on conditions.
6. Most of the serum proteins are denatured and thereby are rendered insoluble (see Section 6.2.2.2).
7. Enzymes (Section 2.5) are inactivated (see Figs. 2.25 and 6.9 and Section 6.3.4).
8. Reactions between protein and lactose occur, Maillard reactions in particular (Section 6.2.3). This involves loss of ''available lysine.''
9. Free sulfhydryl groups are formed. This causes, for instance, a drop of the redox potential (Section 1.2.3).
10. Other reactions of proteins occur (see Section 6.2.2).
11. Casein micelles become aggregated. Aggregation may eventually lead to coagulation (Section 6.2.4).
12. Several changes occur in the fat globule membrane, e.g., in its Cu content.
13. Glycerides are hydrolyzed and interesterified (Section 2.3).
14. Lactones and methyl ketones are formed from the fat (Section 2.3.1).
15. Some vitamins are degraded.
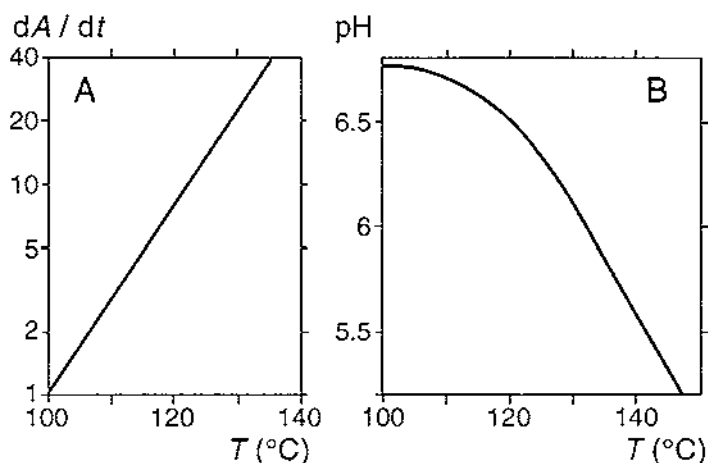
FIGURE 6.1 Acid production in milk during heating as a function of the temperature T. Approximate results. (A) Acidity produced, in mEq $\cdot$ L^{-1} $\cdot$ h^{-1}; (B) pH at room temperature after 30 min heating. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

6.2.1.2 Consequences

Usually, the main effect of heat treatment is the far slower rate of deterioration due to microbial and enzymatic action. The most important other effects are as follows:

a. *Color*. Heating milk at first makes it a little whiter, maybe via change 2 above. At increasing heating intensity the color becomes brown, due to 8 (see Fig. 6.2).

b. *Viscosity* may increase slightly due to 6, and much more due to 11 (if it happens). The latter change especially occurs when concentrated milk is sterilized.

c. The *flavor* (Section 2.6.4) changes appreciably, mainly due to changes 8, 9, 10, and 14.

d. *Nutritive value* decreases, at least for some nutrients, due to 15 and 8 and maybe 10. Examples are given in Table 6.1.

e. Several bacteria can grow faster in heat-treated milk because bacterial inhibitors like lactoperoxidase-H_2O_2-CNS and immunoglobulins are inactivated (nos. 6 and 7). Furthermore, heat treatment may lead to formation of stimulants for some bacteria or inhibitors for still other bacteria. All of these changes greatly depend on heating intensity.

f. Tendency for age thickening and for heat coagulation of concentrated milk may be decreased (Sections 6.2.4 and 16.1.4 and 16.1.6).
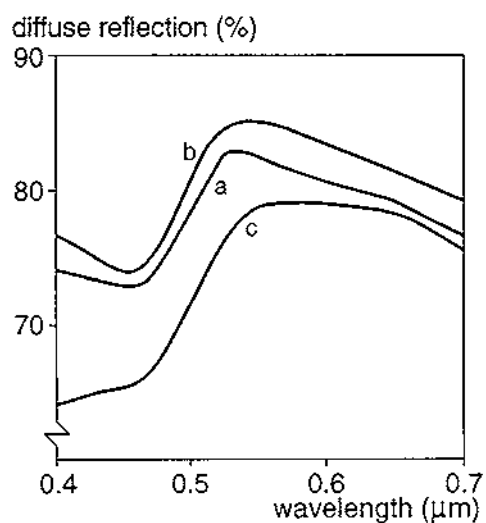
FIGURE 6.2 Diffuse reflection spectra of homogenized milk. (a) Unheated; (b) heated for 30 s at 130°C; (c) heated for 40 min at 115°C. The decreased reflection at short wavelengths causes a brownish color. After H. Radema, Netherlands Institute for Dairy Research (unpublished).

    g. The *rennetability* decreases (Section 21.3.5).
    h. *Creaming tendency* of the milk decreases (Section 3.1.4), mainly caused by 6.
    i. The proneness to *autoxidation* is affected in several ways (Section 2.3.3), mainly due to 12, 9, and 7.
    j. The composition of the surface layers of the fat globules formed during homogenization or recombination is affected by the intensity of heating

TABLE 6.1 Loss in % of Some Nutrients, i.e., Available Lysine and Various Vitamins, Due to Some Heat Treatments of Milk

Heat treatment	Available lysine	Vitamins				
		B_1	B_6	B_9	B_{12}	C
15 s at 75°C	0	5–10	0–5	3–5	3–10	5–20
15 s at 140°C	0	5–15	5–10	10–20	10–20	10–20
20 min at 115°C	5–10	20–40	10–20	20–50	30–80	30–60

Note: Loss of vitamins B_1, B_9, and C depends on the oxygen concentration during heat treatment.

before homogenization, mainly because of change no. 6. This affects some properties of products. For example, the tendency to form homogenization clusters (Section 8.7) is increased.

6.2.2 Reactions of Proteins

Several reactions of side chain groups (and possibly of terminal groups) of proteins can occur at high temperature. Table 6.2 gives examples. Many of these

TABLE 6.2 Possible Reactions of Side Chain Groups of Amino Acid Residues Linked in the Peptide Chain (|) of Proteins at High Temperature

1. $\vdash CH_2 - CONH_2 + H_2O$ $\longrightarrow$ $\vdash CH_2 - COO^- + NH_4^+$
   Asparagine   Aspartic acid

2. $\vdash (CH_2)_2 - CONH_2 + H_2O$ $\longrightarrow$ $\vdash (CH_2)_2 - COO^- + NH_4^+$
   Glutamine   Glutamic acid

3. $\vdash CH_2 - O - PO_3^{2-} + H_2O$ $\longrightarrow$ $\vdash CH_2 - OH + HPO_4^{2-}$
   Phosphoserine   Serine

4. $\vdash CH_2 - SH + OH^-$ $\rightleftarrows$ $\vdash CH_2 - S^- + H_2O$
   Cysteine

5. $\vdash CH_2 - S$
                 $\diagdown$
                     $S - CH_2 \dashv$ $\rightleftharpoons$ $\vdash CH_2 - S^-$
              $+$ $+$
   $\vdash CH_2 - S^-$ $\diagup S - CH_2 \dashv$
                                                       $\vdash CH_2 - S$

6. $\vdash CH_2 - S^- + {}^-S - CH_2 \dashv$ $\longrightarrow$ $\vdash CH_2 - S - S - CH_2 \dashv + 2\ominus$
   Cysteine   Cystine

7. $\vdash CH_2 - S^-$ $\longrightarrow$ $\vdash CH_2 \qquad + HS^-$
   Cysteine   Dehydroalanine

8. $\vdash CH_2 - O - PO_3^{2-}$ $\longrightarrow$ $\vdash CH_2 \qquad + HPO_4^{2-}$
   Phosphoserine   Dehydroalanine

9. $\vDash CH_2 \qquad + HS - CH_2 \dashv$ $\longrightarrow$ $\vdash CH_2 - S - CH_2 \dashv$
   Dehydroalanine   Cysteine   Lanthionine

10. $\vdash (CH_2)_4 - NH_3^+ + H_2C \vDash \quad + OH^- \longrightarrow \vdash (CH_2)_4 - NH - CH_2 \dashv + H_2O$
    Lysine   Dehydroalanine   Lysinoalanine

11. $\vdash CH_2 - (C_3H_3N) - NH^+ + H_2C \vDash \quad + OH^- \rightarrow \vdash CH_2 - (C_3H_3N) - N - CH_2 \dashv + H_2O$
    Histidine   Dehydroalanine   Histidinoalanine

12.[a] $\vdash CH_2 - COOH + H_2N - (CH_2)_4 \dashv \quad \dashrightarrow \quad \vdash CH_2 - CO - NH - (CH_2)_4 \dashv + H_2O$
    Aspartic acid   Lysine   Isopeptide

[a] Reaction also occurs with glutaminic acid residues.

reactions, i.e., 5, 6, 9, 10, 11, and especially 12, can form crosslinks within or between peptide chains; crosslinking reactions may reduce the solubility of the protein. The rate of most of the reactions and their equilibrium states are poorly known. Besides reactions 1 and 2, which occur readily, and 4–6, which may occur upon denaturation of the protein, most of the reactions involved require high temperatures (sterilization). Since casein contains phosphoserine, dehydro-alanine can readily be formed (reaction 8, but also from reaction 7). All in all, in milk most of the reactions considered may occur, though only very small amounts of, for instance, lysinoalanine (reaction 10) are formed unless the pH is very high; lysinoalanine might be toxic because its ingestion can cause changes in the kidney of rats (not observed in humans).

As stated above, a high temperature is needed for most of the reactions to occur. It is not always necessary for the reaction itself, but anyhow it is for the unfolding of the peptide chain (denaturation); thereby the groups involved become exposed and available for reactions. Most reactions proceed faster at higher pH, but not 3 and 11.

6.2.2.1 Reactions of Thiol (Sulfhydryl or –SH) Groups

The –SH group of cysteine is very reactive in the ionized form (reaction 4). In the peptide chain its pK is about 9.5 at 25°C. This means that at pH 6.1, 6.4, and 6.7, on average 0.04%, 0.08%, and 0.16% of the group, respectively, is dissociated. Consequently, reaction 4 is strongly dependent on pH. Of course, before this reaction can occur the peptide chain must be unfolded, unless the thiol group would be on the outside of the native molecule, which is exceptional. Heat treatment of milk such that denaturation of serum proteins occurs therefore results in a considerable increase in the number of reactive thiol groups. Upon such heating, mainly reaction 5 proceeds, thus shifting the position of –S–S– linkages involved. The disulfide interchange may considerably affect the conformation of a protein molecule. Reaction 6, especially its direction, closely depends on the redox potential because this crosslinking reaction occurs by oxidation. In milk, the reaction is usually toward the right-hand side.

Formation of H_2S (reaction 7, but presumably also other reactions) causes a cooked or even ''gassy'' flavor to develop in milk. As a rule, no more than, say, 1% of the thiol groups reacts. The formed dehydroalanine residue readily reacts, according to reactions 9, 10, and 11.

Table 2.18 shows in what milk proteins –S–S– and –SH groups occur; the immunoglobulins also contain much cystine. The main contributor to the groups probably is β-lactoglobulin, due to its high concentration in milk and its free thiol group. Upon heating, the thiol group becomes very reactive; because of that, irreversible changes in the molecule occur. As far as the formation of H_2S is concerned, a part of the fat globule membrane protein is by far the most active

agent, at least 10 times more active than β-lactoglobulin; heating of skim milk hardly produces H_2S, heating of cream far more (see also Section 3.1.1).

6.2.2.2 Denaturation of Serum Proteins

Globular proteins exhibit unfolding of their peptide chain at high temperature, say 80°C, although marked variation in the temperature needed is observed among proteins. As mentioned above, reactions occurring in or between side groups in the peptide chain at the prevailing temperature may then prevent refolding of the peptide chain into its original, i.e., native, conformation. In other words, the protein remains denatured. (Generally, the peptide chain does not remain fully unfolded but assumes some other coiled-up conformation.) As a result, most proteins lose their biological activity, e.g., as an enzyme or as an antibody. Generally, they also become less soluble. Otherwise, the changes may be mild and the nutritive value is rarely impaired.

These changes occur with the globular serum proteins in milk, namely, β-lactoglobulin, α-lactalbumin, serum albumin, and the immunoglobulins (as well as most minor serum proteins). The proteose-peptones, like the caseins, are not denaturable. Heat denaturation of β-lactoglobulin has been studied in some detail. At high temperature, the free thiol group becomes exposed and it reacts with one of the –S–S– groups (Table 6.2, reaction 5), generally of another molecule, whereby both molecules become bonded, forming a dimer. In this way, also trimers, tetramers, etc., are formed, but the aggregates may remain fairly small and soluble. Depending greatly on conditions, especially pH, but also ionic composition and temperature, further aggregation may now occur, resulting in large insoluble particles; at high concentrations (over 10% or 12%) a gel may even be formed. Much the same happens with the other proteins, although some of them lack free thiol groups. This implies that either the reaction scheme must be different or that a free thiol group of another protein (β-lactoglobulin, bovine serum albumin, immunoglobulins) is involved. When heating a solution of serum proteins, e.g., whey, at various pH, it is observed that they only become insoluble at low pH (see Fig. 6.3A). Also, heating at neutral pH and acidification after cooling leads to insolubility. Insolubility is enhanced at high Ca^{2+} activity.

When milk is heated, the reactions are partly different. β-Lactoglobulin reacts with κ-casein, which is located at the outside of the casein micelle. In this reaction, –S–S– interactions as well as other bonds play a part. The result is that the casein micelles become ''covered'' by a layer of denatured β-lactoglobulin. However, this greatly depends, again, on pH, as illustrated in Figure 6.3B. At high pH, after heating and cooling, a complex of β-lactoglobulin and κ-casein is found in solution. The other serum proteins exhibit similar reactions, and may become associated with the casein micelles or form separate aggregates. Part of the denatured serum protein becomes associated with the milk fat globules. Serum
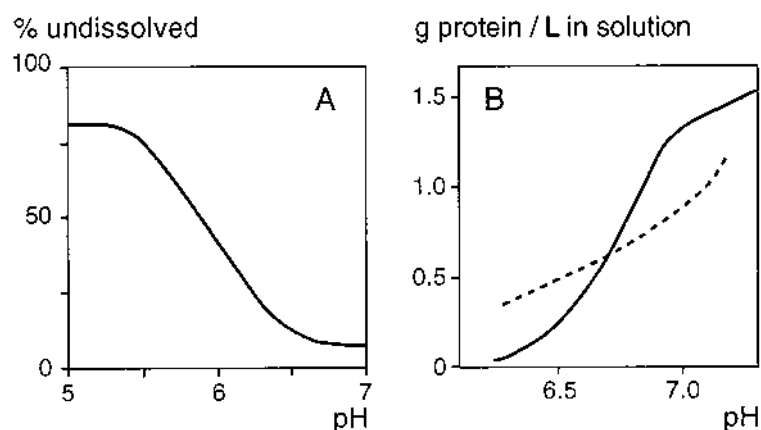
FIGURE 6.3 Influence of pH on the effects of heating on proteins. (A) Percentage of the proteins that become undissolved after heating whey for 10 min at 80°C. (B) Amount of protein that remains in solution, i.e., not associated with the casein micelles, after heating milk (——) or serum protein free milk (---) at 140°C.

protein denaturation causes a slight increase in milk viscosity because the voluminosity of the proteins increases, e.g., from 1 to 3 ml per g protein. When heating milk, cooling it, and then acidifying it to pH 4.6, the isoelectric pH of casein, the denatured serum proteins become associated and precipitate with the casein.

Kinetics of the denaturation reaction are given in Figure 6.4 and it is seen that the various serum proteins differ in heat sensitivity; see also Figure 6.9E and Section 6.3.2.

6.2.2.3 Degradation

Upon heating at high temperature, cleavage of various parts of the molecules may occur. Such cleavage has been mainly demonstrated to occur in casein. An example is dephosphorylation of caseinate; it is uncertain as to what extent the phosphate cleavage occurs by hydrolysis (reaction 3) or β elimination (reaction 8). Furthermore, severe heat treatment cleaves peptide chains, yielding soluble peptides. N-acetyl neuraminic acid and possibly other carbohydrates may be cleaved from casein as well. For example, in a study on heating caseinate solutions, a treatment of 20 min at 120°C caused 2.7%, 0.9%, and 1.1% of the nitrogen and 9.5%, 7.5%, and 14.4% of the organic phosphorus of α_s-, β-, and κ-casein, respectively, to become soluble (i.e., not precipitated with the casein at pH 4.6). After treatment at 135°C for 1 h all of the P and 15% of the N became ''soluble.''
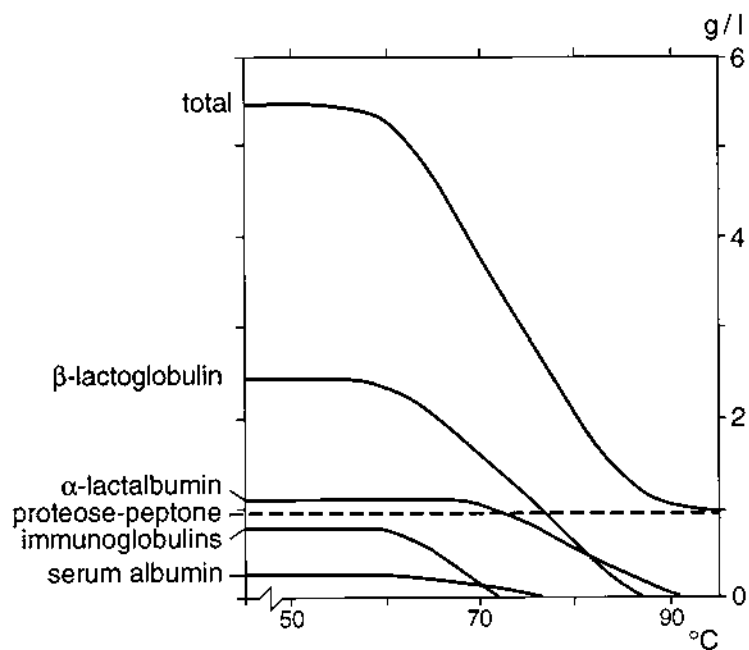
FIGURE 6.4 Effect of heating milk for 30 min at various temperatures on quantity of serum proteins that remain dissolved after cooling and acidification to pH 4.6. Mainly after B.L. Larson and G.D. Rolleri, *J. Dairy Sci.* **38** (1955) 351.

6.2.3 Reactions of Lactose

During heating of milk, lactose suffers reactions that have important consequences for the milk. For example, resultant changes in flavor, color, nutritive value, and pH may occur. As is discussed in Section 2.1.1, lactose is a reducing sugar that reacts with amino groups (in milk mainly supplied by lysine residues) in the very important Maillard reaction. Besides, lactose may isomerize into other sugars. Figure 6.5 schematically shows the initial stage of the reactions.

The isomerization reaction (1) thus parallels the Maillard reaction (2), and also proceeds in the absence of amino groups, though more slowly; amino groups supposedly catalyze reaction (1). Lactulose (a disaccharide of galactose and fructose; see Fig. 2.1) is formed in fairly large quantities, from 300 to over 1000 mg $\cdot$ L^{-1} (3 mmolar) in sterilized milks. Epilactose (disaccharide of galactose and mannose) is only formed in trace amounts. In principle, all of these isomerization reactions are reversible. Furthermore, the fructose moiety of lactulose may be split up into formic acid and a C_5 compound or changed to a C_6 compound, while

epilactose

(1) lactose ⇌ lactulose ⟶ galactose + formic acid + C$_5$ compound

galactose + C$_6$ compound

(2) lactose + R-lysine ⇌ R-lactulosyllysine ⟶ galactose + R-lysine + formic acid + IMP

galactose + R-lysine + IMP
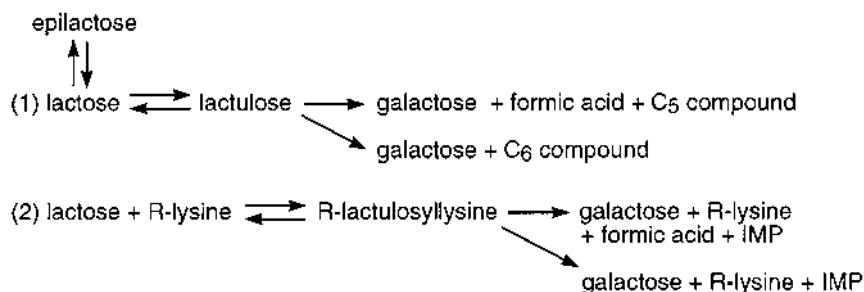
FIGURE 6.5 Simplified scheme of reactions occurring in the initial stage of the breakdown of lactose during the heating of milk (sterilization temperatures). IMP, intermediate Maillard products.

galactose is left. Among C$_5$ compounds detected are furfural, furfuryl alcohol, deoxyribose, and 3-deoxypentulose. The last two are unstable compounds. Formation of C$_6$ compounds includes hydroxymethyl furfural (HMF), in addition to other, unidentified products. These ensuing reactions are irreversible. The formic acid formed is primarily responsible for the increased acidity of heated milk (alterations in dynamic equilibria between the salts in milk due to heating cause a largely temporary decrease in pH; see Section 2.2.4).

It is only in a later stage of the heating of milk that the Maillard reaction (2) manifests itself in changes in flavor and color. In its initial stage, it is a reaction between lactose and a lysine residue. Through a number of steps, the more or less stable intermediate product lactulosyllysine-R is formed; reactions with lactulose and epilactose lead to a similar product. From the fructose moiety of lactulosyllysine, HMF can be formed; galactose is left, whereas the lysine residue is released again. (Essentially, lysine thus is active as a catalyst.) Amounts of HMF produced are on the order of some tens of micromoles per liter, i.e., much less than lactulose. Also other, low molar mass reaction products are formed from the intermediate lactulosyllysine, including acetol, methylglyoxal, maltol, and several other aldehydes and ketones. To be sure, all of these compounds may be formed in fairly small amounts, but they are important with reference to flavor development and because of their reactivity. At a later stage of the Maillard reaction, polymerization reactions of amino compounds with substances like HMF and furfural occur. The latter substances also polymerize without amino compounds being present. The polymers formed are called melanoidins. They cause the milk to have a brown color. The molecular structure of the melanoidins is very intricate and has not been sufficiently elucidated. Advanced Maillard reactions may cause crosslinking of proteins.

Of all reactions occurring, the Maillard reaction in particular is an intricate

one, and much about it is unclear. The various reactions involved affect each other, an important factor when studying this kind of reaction. For example, the drop in pH, mainly resulting from formic acid production, decreases the rate of isomerization as well as of the Maillard reactions. The reactions proceed faster at a higher temperature (Q_{10} is 2–3), but the temperature dependence of the reactions as shown in, for instance, Figure 6.9 probably is different for every reaction. Once the Maillard reaction gets started, it proceeds at an appreciable rate even at a lower temperature, as can be noticed during storage of evaporated milk. Of course, the composition of milk and milk products also affects the reactions, not only because of the concentration of reactants but because of the possible presence of components that are active as a catalyst. Therefore, it is hard to predict the effects resulting from a change in milk composition.

Summarizing the above, it may be stated that in not too intensely heated milk, lactose will predominantly be decomposed by isomerization reaction (1), whereas a small part will be degraded through later stages of the Maillard reaction (2). Only during very intense heating, and also in further stages of the reactions, the Maillard reaction plays a part. Resultant manifestations are changes in flavor, development of brown color by formation of melanoidins, and a certain loss in nutritive value caused by rendering lysine unavailable (see Table 6.1).

6.2.4 Heat Coagulation

Casein does not show heat denaturation as suffered by globular proteins. But at a very intense heat treatment it can aggregate under certain conditions, especially if in micellar form (general aspects of the casein micelle stability are discussed in Section 3.2.3). Under practical conditions, the reaction can manifest itself as coagulation during sterilization. The coagulation may become visible when large aggregates have emerged or by formation of a gel. The time needed for this to occur is called the *heat coagulation time* (HCT).

The heat coagulation of milk is an intricate phenomenon. This is because several interactions and conditions play a role. The most important variable is pH. The initial pH of milk considerably affects HCT, i.e., the lower the pH, the lower the temperature at which coagulation occurs. Figure 6.6A gives an impression. By and large, at constant temperature the rate of coagulation increases with decreasing pH. But often a local minimum in the heat coagulation time occurs near pH 6.8–6.9 (called the pessimum pH) and a local maximum near pH 6.6 (called the optimum pH). Milk that behaves like this is referred to as milk of type A; if it does not it is milk of type B, as shown in Figure 6.6B. Type A milk is by far the most common. There is considerable variation in heat stability among lots of milk. These are not completely understood; in many regions a seasonal effect is observed (see, e.g., Fig. 1.9D), partly caused by variation in the natural urea level.
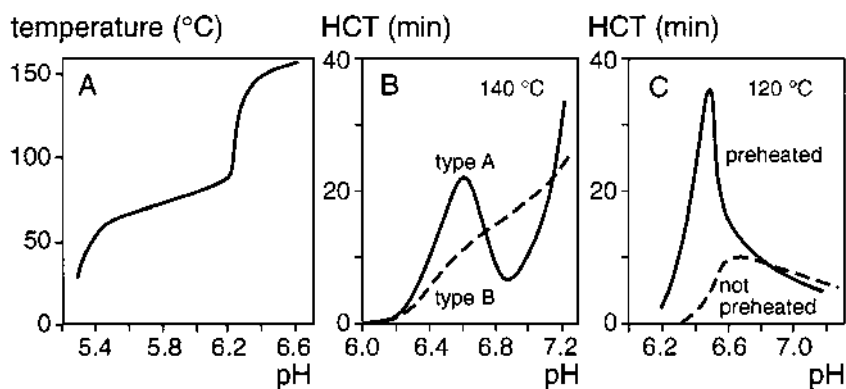
FIGURE 6.6 Heat coagulation of milk as a function of the initial pH. (A) Temperatures at which coagulation starts at fairly rapid warming of the milk (approximate results). (B) Heat coagulation time at 140°C of two different samples of fresh milk. (C) HCT at 120°C of evaporated skim milk, with or without preheating of the milk before concentration.

Apart from what happens near the pessimum pH, coagulation only occurs when the pH of the milk has become low, i.e., pH < 6.2 (the pH is lowered during heating; see Fig. 6.1). Nevertheless, the aggregation generally is irreversible, i.e., the aggregates formed cannot be redispersed by increasing the pH. It thus appears that the aggregates are held together by chemical (covalent) crosslinks. All the same, colloidal interaction forces are essential because the micelles have always to come close enough before crosslinking can occur. The following are factors that determine the colloidal interaction:

a.   κ-Casein (provides steric and electrostatic repulsion)
b.   pH (affects electrostatic repulsion)
c. Ca^{2+} activity (Ca^{2+} may form salt bridges, affects electrostatic repulsion)

See also Section 3.2.3, especially Figure 3.17.

The pH decrease that occurs during the heating of milk is an essential factor in the heat coagulation of milk. The initial decrease in pH is mainly caused by ''precipitation'' of calcium phosphate (Section 2.2.4) and the further decrease by production of formic acid from lactose (Section 6.2.3). The rate of pH decrease largely determines the rate of coagulation. The influence of a number of factors on heat coagulation is reflected in the rate of pH decrease. Often, coagulation only occurs after milk pH < 6.2, so that a higher initial pH would require a longer time to reach a sufficiently low pH for heat coagulation to occur. As stated

above, heat coagulation is, however, not simply equal to coagulation by acid. Obviously, additional reactions play a part.

The following model may explain most observations on heat coagulation of milk. There are two different reactions that may cause coagulation. The first is *colloidal aggregation*, in which Ca^{2+} ions play a crucial role, presumably via Ca bridging. The coagulates formed can be dissolved by adding Ca-chelating agents (unless a fairly long heating time was needed for coagulation to occur). The reaction is second order. Its rate is not strongly dependent on temperature. It depends very much on Ca^{2+} activity. For a lower pH, a_{Ca}^{2+} of milk is higher, as seen in Figure 2.9. Heating itself causes two effects—a lower pH and a lower a_{Ca2+}—at the same pH (Fig. 2.9). This means that during heating of milk, a_{Ca}^{2+} does not greatly alter because both effects roughly compensate each other.

The second reaction is a *chemical crosslinking*, although the crosslinks involved have not been identified. (The problem is that at high temperature several types of crosslinks are formed, also inside casein micelles and between micelles that had already formed aggregates.) The reaction is much faster at higher temperature ($Q_{10} \approx 3$) and it very much increases in rate as the pH decreases. This means that the chemical reaction often overtakes the colloidal one, at least during heating of untreated milk: The first reaction proceeds at a slow rate, until the pH has reached a value, generally about 6.2 as measured at room temperature, where the second reaction becomes quite fast. The HCT is then largely determined by the rate of acid production during heating.

Another essential point is *depletion of κ-casein* from the micelles, making them less ''hairy.'' This may be explained by the results in Figure 6.3B, where we will first consider the curve in the absence of serum proteins. At high temperatures, more protein is outside the micelles at a higher pH and this concerns for the most part κ-casein. There is an equilibrium between κ-casein in the micelles and in solution, which shifts toward the solution with increasing pH. It is also seen that the change is more pronounced in the presence of serum proteins, occurring over a narrower pH range. As mentioned (Section 6.2.2.2), β-lactoglobulin and κ-casein react at high temperature. At high pH (>6.7), this occurs for the most part in solution, lowering the concentration of κ-casein in solution, thereby disturbing the partition of κ-casein between solution and micelles. The result is that more κ-casein leaves the micelles. At a low pH (<6.7), the opposite will happen. Consequently, the state of depletion of the casein micelles very steeply depends on pH. Depleted micelles will be much less stable to aggregation than those with κ-casein hairs. Once fully depleted, the micelles would be more stable at higher pH because that implies a higher negative charge; the same holds for micelles not depleted of κ-casein.

The relations discussed lead to the model given in Figure 6.7. At fairly high initial pH, the micelles become depleted of κ-casein and the colloidal reaction is rate determining for coagulation. If the initial pH is higher, the micelles are suffi-
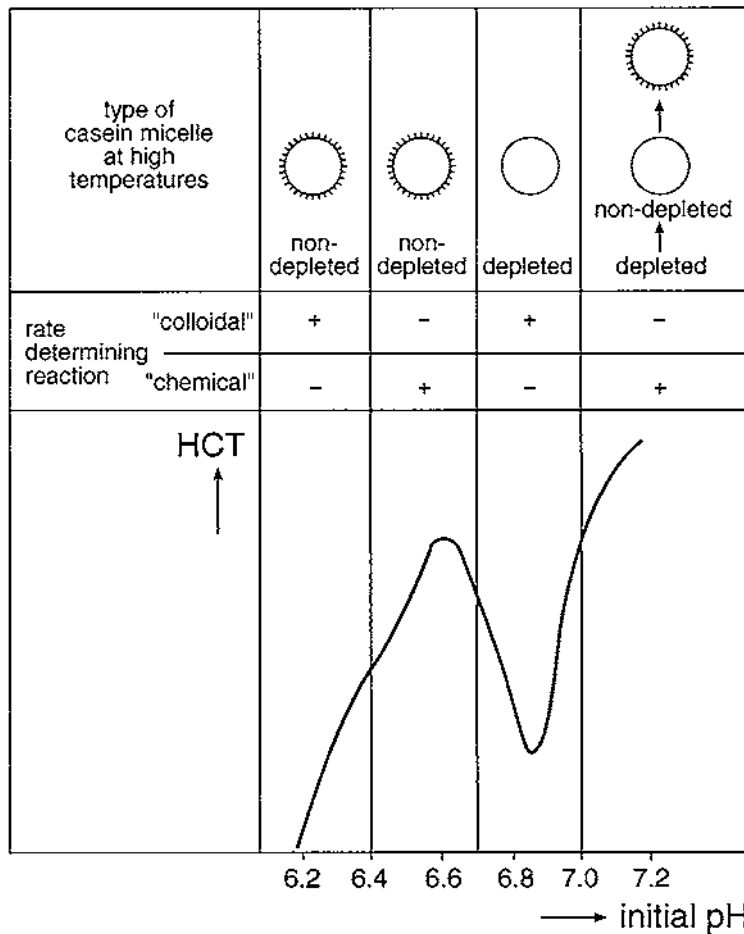
FIGURE 6.7 Model for the effect of initial pH on the type of casein micelle emerging at high temperature and thereby on the heat coagulation time (HCT) of milk.

ciently stable (high electric charge, very low $a_{Ca^{2+}}$) to allow the pH to decrease until they become hairy again; presumably, the complex between β-lactoglobulin and κ-casein precipitates onto the micelles, providing steric repulsion. Now the pH is low enough for the chemical crosslinking to become rate determining, as is also the case if the initial pH is around 6.6. At very low pH, the Ca^{2+} activity is so high that the colloidal reaction is fastest.

In practice, heat coagulation of milk is rarely a problem, but *concentrated milk* (e.g., evaporated milk) may coagulate during sterilization. It is far less stable than untreated milk, as seen by comparing parts B and C of Figure 6.6; note the difference in temperature. This is primarily due to the higher concentration of casein: A second-order reaction proceeds faster at a higher concentration for the same rate constant. Moreover, other conditions are changed. Although for the most part the same mechanisms act during heat treatment, there are important differences in consequences between milk and concentrated milk.

To begin with, the heat stability of concentrated milk is considerably increased in the acid pH range if the original milk had been preheated (preheating has little, if any, effect on the heat stability of plain milk). This is explained as follows. In nonpreheated concentrated milk, the serum proteins are in the native state. During warming to 120°C, the serum proteins become denatured and in the acid pH range they strongly aggregate. Due to the high concentration of serum proteins (they have also been concentrated) a gel is formed. In other words, the casein micelles become incorporated in a serum protein gel. At higher pH, the denatured serum proteins remain dissolved and no serum protein gel is formed. In concentrated milk made from preheated milk, the serum proteins have already been denatured and have become associated with the casein micelles. During preheating of the nonevaporated milk, formation of a gel is not possible because the serum protein concentration is too low, and in the concentrated milk it will not occur because the serum proteins have already been denatured.

Furthermore, in concentrated milk the increase in stability from pH 6.2 to pH 6.5 is, as in milk, ascribed to the decreasing Ca^{2+} activity. The decrease of the stability at pH > 6.6 is, again as in milk, caused by dissociation of κ-casein as a result of which depleted micelles remain that are susceptible to Ca. If the pH increases to >7.0, the stability does not increase again as happens in milk (Fig. 6.6B). This is due to the increased salt concentration, since merely rising salt concentration in unconcentrated milk has virtually the same effect on heat stability at high pH. Presumably, during heating so much calcium phosphate associates with the micelles as to make them very unstable. This is somewhat comparable to the Ca^{2+}-induced coagulation of depleted micelles in unconcentrated milk, but the reaction with calcium phosphate is much faster.

There is another complication, which is that for the same reaction rate the coagulation time can be very different under different conditions. This is best illustrated for concentrated skim milk, as is done in Figure 6.8, which compares heat coagulation at a pH near the optimum (about 6.5) and at a lower and a higher value; cf. Figure 6.6C. Near pH 6.3, the micelles aggregate to form open clusters, which will soon fill the whole volume, whereby a gel is formed; this is discussed as fractal aggregation in Section 3.2.3.5. In agreement with the open structure of the aggregates, the turbidity does not increase greatly, but the (apparent) viscosity does: the volume fraction of material greatly increases. At pH 6.8, the
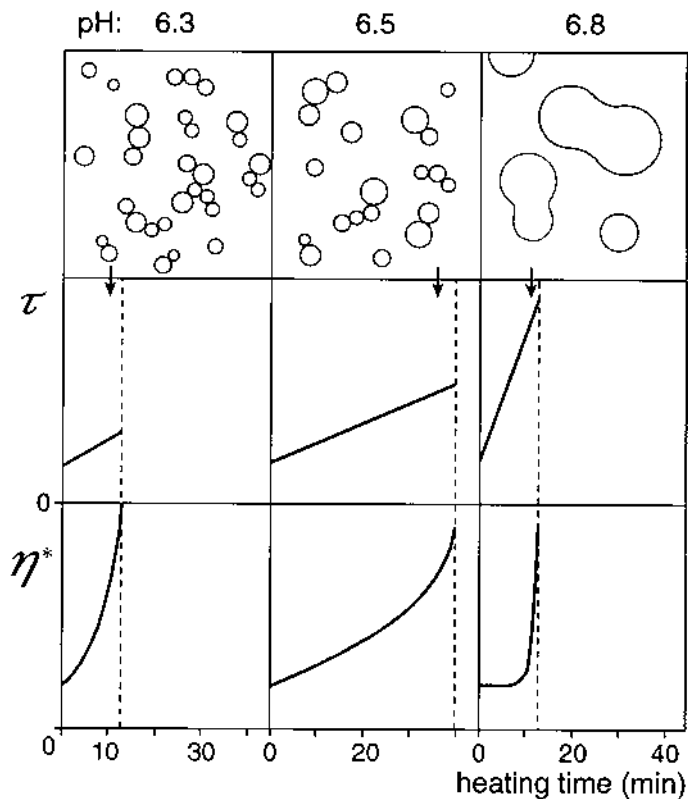
FIGURE 6.8 Heat coagulation at 120°C of concentrated skim milk at various initial pH. The upper row shows the appearance of the casein micelles (derived from electron micrographs) at the moments indicated by arrows, i.e., shortly before heat coagulation. The HCT is indicated by a vertical broken line. The second row gives the turbidity (τ) as a function of heating time, the lowest row the apparent viscosity (η^*). τ and η^* were determined in situ, i.e., at 120°C. Approximate, after results by J.A. Nieuwenhuijse et al., *Neth. Milk Dairy J.* **45** (1991) 193–224.

depleted micelles tend to fuse into larger ones upon aggregation. This leads to a large increase in turbidity but not to a higher viscosity: the volume fraction does not alter. Only in the final stages do the aggregates become of irregular shape and form a gel. At pH 6.3, about 10 micelles would form an aggregate of critical size for gelation, whereas the large aggregates formed at pH 6.8 would have been made up of about 1000 micelles. Nevertheless, the HCT is the same, which means that the aggregation reaction rate ($-\mathrm{d}N/\mathrm{d}t$, where N is the particle

number) was much greater at the higher pH. At pH 6.8, near the maximum HCT, the reaction proceeds for the most part as at pH 6.3, although some fusion of micelles occurs. However, the reaction is slower because of the higher pH, presumably due to stronger electrostatic repulsion.

Most of the effects on heat coagulation of milk can be explained by using the model described above. For example, we have:

a. *Protein composition*. Its effect specifically concerns the ratio between κ-casein and β-lactoglobulin. The larger the amount of β-lactoglobulin, the higher the maximum HCT and the deeper the minimum. This is explained by β-lactoglobulin enhancing the dissociation of κ-casein at pH > 6.7, which results in formation of more strongly depleted micelles. The higher maximum at pH 6.6 may result from an increased association of β-lactoglobulin with the micelles, which may enhance colloidal repulsion.

b. *Urea content*. The higher it is, the stabler the milk toward heat coagulation, at least near the optimum pH. This can partly be explained by urea slowing down the pH decrease, but there are also other effects. Incidentally, urea does not affect the heat stability of concentrated milk (unless urea concentration is very high).

c. *Salt composition*. Its main influence is through the calcium and phosphate contents. The addition of a certain salt to milk can strongly disturb all salt equilibria involved (Section 2.2.4). Addition of calcium and phosphate to milk to concentrations as in concentrated milk causes its heat stability at pH > 6.8 to be equal to that of concentrated milk, i.e., zero.

d. *Fat*. Fat in itself does not affect heat stability. This is no surprise, considering heat coagulation to be a coagulation of casein micelles. It becomes different if casein enters the fat globule–plasma interface, as occurs in homogenization (see Fig. 8.7). This makes the fat globules behave like large casein micelles and they coagulate along with the micelles. As a result, in products like cream and concentrated milk, homogenization tends to cause a lower heat stability.

6.3 HEATING INTENSITY

The intensity of heating follows from the duration (t') of heating and the temperature (T). Figure 6.9 gives several examples. The effects of a certain combination of t' and T will differ because they depend on the reaction considered, e.g., inactivation of a certain enzyme or formation of Maillard products. After all, certain reactions occur fairly quickly at relatively low temperature, whereas others need a much higher temperature before having an appreciable effect. The dependence
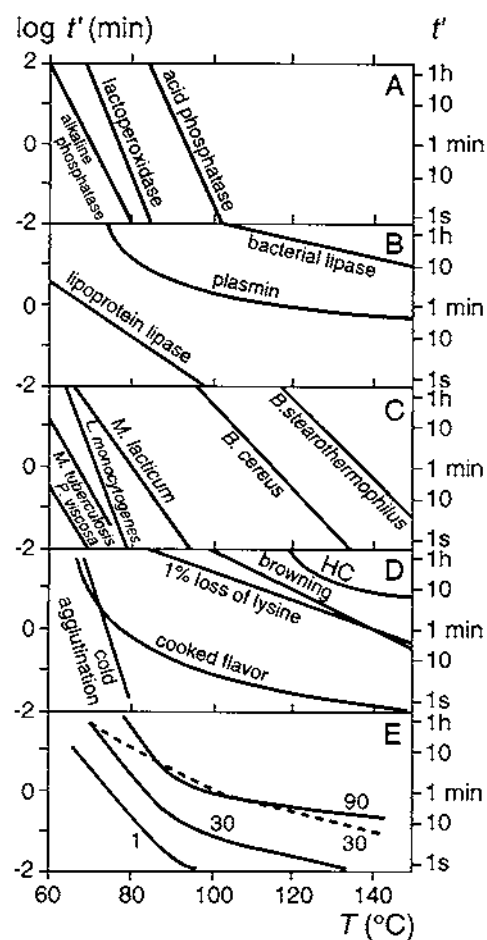
FIGURE 6.9 Combinations of temperature (*T*) and time (*t′*) of heating of milk that cause (A, B) inactivation (reduction of activity to about 1%) of some milk enzymes and a bacterial lipase; (C) the killing (reduction of the count to 10^{-6}) of strains of the bacteria *Pseudomonas viscosa, Mycobacterium tuberculosis, Listeria monocytogenes*, and *Microbacterium lacticum*, and of spores (10^{-4}) of *Bacillus cereus* and *B. stearothermophilus*; (D) visible heat coagulation (HC), a certain degree of browning, decrease in available lysine by 1%, a distinct cooked flavor and inactivation of cold agglutination; (E) insolubilization of 1%, 30%, and 90% of the β-lactoglobulin, and of 30% of the α-lactalbumin (---). Approximate results after various sources.

of the reaction rate on temperature varies widely among reactions, which explains why at a certain combination of t' and T (e.g., 15 min at 110°C) reaction A may have advanced further than reaction B, whereas it is just the opposite at another combination, e.g., 10 s at 140°C. This is illustrated in Figure 6.10.

6.3.1 Processes of Different Intensity

In classifying heating processes on the basis of their intensity, special attention is usually paid to the killing of microorganisms and to the inactivation of enzymes (see also Figure 6.9). The following are customary processes.

a. *Thermalization*. This is a heat treatment of lower intensity than low pasteurization, usually 20 s at 60–69°C. The purpose is to kill bacteria, especially psychrotrophs, as several of these produce heat-resistant lipases and proteinases that may eventually cause deterioration of milk products. Except for the killing of many vegetative microorganisms, thermalization causes almost no irreversible changes in the milk.

b. *Low pasteurization*. This is a heat treatment of such intensity that the enzyme alkaline phosphatase (EC 3.1.3.1) of milk is inactivated. It may be realized by heating for 30 min at 63°C or for 15 s at 72°C. Almost all pathogens that can be present in milk are killed; it specifically concerns *Mycobacterium tuberculosis*, a relatively heat-resistant organism that formerly was among the most serious pathogens. All yeasts and molds and most, but not all, vegetative bacteria are killed. Some species of *Microbacterium* that grow slowly in milk are not killed (Fig. 6.9C). Furthermore, some enzymes are inactivated but by no means all of them. Flavor of milk is hardly altered, little or no serum protein is denatured, and cold agglutination and bacteriostatic properties remain virtually intact. A more intense heat treatment is, however, often applied (e.g., 20 s at 75°C; see Section 14.1.1). This causes, for instance, denaturation of immunoglobulins (hence decrease in cold agglutination and in bacteriostatic activity) and sometimes a perceptible change in the flavor of milk.

c. *High pasteurization*. This is a heat treatment such that activity of the enzyme lactoperoxidase (EC 1.11.1.7) is destroyed, for which 20 s at 85°C suffices. However, higher temperatures, up to 100°C, are sometimes applied. Virtually all vegetative microorganisms are killed but not bacterial spores. Most enzymes are inactivated but milk proteinase (plasmin) and some bacterial proteinases and lipases are not or not fully. Most bacteriostatic properties of the milk are destroyed. Denaturation of part of the serum proteins occurs. A distinct cooked flavor develops; a gassy flavor if it concerns cream. There are no significant changes in nutritive value, with the exception of a loss of vitamin C. The stability of the product toward autoxidation of fat is increased. All the same, only a few irreversible chemical reactions occur.

d. *Sterilization*. This heat treatment is meant to kill all microorganisms, including the bacterial spores. To that end, 30 min at 110°C (in-bottle sterilization), 30 s at 130°C, or 1 s at 145°C usually suffices. The latter two are examples of so-called UHT (ultra-high-temperature, short time) treatment. Besides, the effects of all such heat treatments are different. Heating for 30 min at 110°C inactivates all milk enzymes, but not all bacterial lipases and proteinases fully; causes extensive Maillard reactions, leading to browning, formation of a sterilized milk flavor, and some loss of available lysine; reduces the content of some vitamins; causes considerable changes in the proteins including casein; and decreases the pH of the milk by about 0.2 unit. Heating for 1 s at 145°C does not inactivate all enzymes, i.e., plasmin hardly and some bacterial lipases and proteinases not at all, and therefore such heat treatment is rarely applied; chemical reactions hardly occur, most serum proteins remain unchanged, and only a weak cooked flavor develops.

e. *Preheating*. This may mean anything from very mild to quite intense heating. It mostly concerns heating intensities anywhere between low pasteurization and sterilization.

6.3.2 Kinetic Aspects

As is discussed in Section 6.2.1, at high temperature numerous chemical reactions occur in milk. The rate of the reactions and the temperature dependence of the rate are variable. Some aspects of the reactions will now be discussed, especially those of importance to the heat denaturation of globular proteins. The latter group of reactions is of great importance because of the consequences involved, including insolubilization of serum proteins, inactivation of enzymes and of immunoglobulins, killing of bacteria and their spores. Denaturation is briefly discussed in Sections 2.4.1.5 and 6.2.2.

In the present discussion it concerns irreversible changes, but denaturation as such (i.e., the unfolding of the peptide chain, which becomes, as it were, ''loosened by vibration'' due to thermal motion) is reversible. The unfolding as caused by the high temperature exposes reactive side groups that now may indeed react. This often leads to irreversible changes (most of these reactions are discussed in Section 6.2.2). The rate at which insolubilization or inactivation occur will largely be determined by the rate of denaturation.

A first-order reaction equation is usually used for denaturation of protein, inactivation of enzymes, and killing of bacteria and spores. We thus have

$$- \, dc/dt = Kc \tag{6.1}$$

where c is concentration, t is time, and K is rate constant. Often, but not always

(see below), this means of calculation appears to be a permitted approximation. Integration of Equation 6.1 yields

$$\ln (c_0/c) = Kt \tag{6.2}$$

where c_0 is the original concentration.

Parameter t' is used for referring to the duration of heating needed to secure a certain effect, e.g., 99% inactivation of an enzyme or 1% of the lactose present converted into lactulose. We thus have

$$t' = \ln (c_0/c')/K \tag{6.3}$$

A particular t' is that for which $c' = c_0/10$; this decimal reduction time D is given by

$$D = (\ln 10)/K \approx 2.3/K \tag{6.4}$$

By using Equations 6.3 and 6.4, a given t' or D can be converted to values for other desirable heating intensities: the number of decimal reductions is proportional to t'.

A reaction equation referring to zero-order kinetics:

$$dc/dt = K \tag{6.5}$$

is commonly used if it concerns formation of a substance that initially is absent. This may be allowed for initial reaction steps, e.g., in the Maillard reaction for the first few per cents of the reducing sugar having reacted with the available lysine residues. Of course, K now depends on the initial concentration of these reactants. Integration of Equation 6.5 yields

$$c = Kt + c_0 \tag{6.6}$$

where often the "blank" value $c_0 \approx 0$. The time needed to arrive at a certain conversion now is proportional to c.

The temperature dependence of a reaction is mostly assumed to follow an Arrhenius relationship. We thus have

$$K(T) = K_0 \exp(-E_a/RT) \tag{6.7}$$

where T = absolute temperature, K_0 = the assumed rate constant at $E_a = 0$ (or $T = \infty$), E_a = the so-called molar activation energy (in $J \cdot mol^{-1}$), and R = the gas constant ($8.314 \ J \cdot mol^{-1} \cdot K^{-1}$).

[*Note*: It is more correct not to use the activation energy E_a (according to Arrhenius), but the activation free energy $\Delta G^{\ddagger}$ (according to Eyringh). Since $G = H - TS$, this leads to a temperature dependence of the reaction rate of the form

$$K(T) \propto \exp(-\Delta H^{\ddagger}/RT) \exp (\Delta S^{\ddagger}/R) \tag{6.8}$$

TABLE **6.3** Typical Examples of the Temperature Dependence of Some
Reactions

Type of reaction	Activation energy[a] (kJ · mol^{-1})	Q_{10} at 100°C
Many chemical reactions	80–125	2–3
Many enzyme-catalyzed reactions	40–60	1.4–1.7
Autoxidation of lipids	40–100	1.4–2.4
Maillard reactions	100–180	2.4–5
Heat denaturation of proteins	200–600	6–175
Enzyme inactivation, e.g.,	450	50
Killing of vegetative bacteria	200–600	6–175
Killing of spores	250–330	9–17

[a] Often an apparent or average activation energy because it mostly concerns a number
of different ensuing reactions.

where $\Delta H^{\ddagger}$ is the activation enthalpy (which is almost equal to E_a) and $\Delta S^{\ddagger}$ the
activation entropy. For most reactions $\Delta S^{\ddagger}$ is small, but not for denaturation of
proteins, since the unfolding of the peptide chain causes a large increase in en-
tropy.]

The temperature dependence is often expressed as the Z value, i.e., the
temperature rise needed to increase the reaction rate by a factor of 10. Conse-
quently,

$$K(T + Z)/K(T) \equiv 10 \tag{6.9}$$

Or it is expressed as Q_{10}, which is defined by

$$Q_{10} \equiv K(T + 10)/K(T) \tag{6.10}$$

In practice we often make curves as shown in Figure 6.9, where log t' is plotted
against the temperature. Usually, fairly straight lines are obtained. Such curves
are very informative, e.g., the optimum combination of temperature and duration
of heating may readily be detected from them. After all, on the one hand it is
desired to kill the bacteria involved and to inactivate enzymes, whereas on the
other hand undesirable changes, such as formation of color and flavor substances,
should be greatly restricted. The required standards can often be approached,
since most of the desirable changes depend far more strongly on the temperature
than most of the undesirable changes do; this is illustrated in Table 6.3. In all
of these cases, it should be known to which extent of inactivation, killing, etc.,
the given t' values correspond; such statements as ''time needed for inactivation
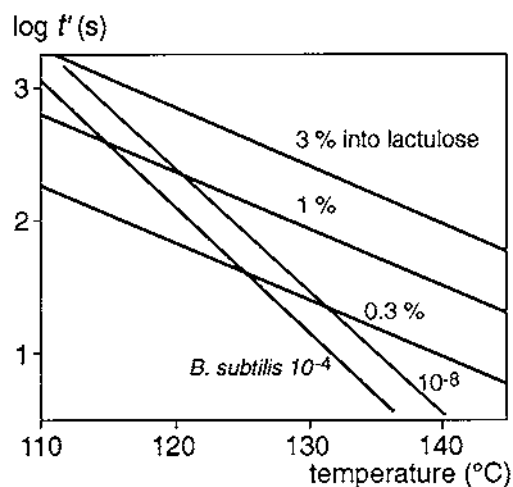
FIGURE 6.10 The time needed (*t'*) at various temperatures to convert certain percentages of lactose to lactulose, and to obtain a certain extent of killing of *Bacillus subtilis* spores.

of peroxidase'' are inadequate. An example is given in Figure 6.10. It refers to the killing of spores of *Bacillus subtilis* (a potential spoilage organism in sterilized milk) and to the formation of lactulose. To be sure, lactulose as such, present in milk in small quantities, is of little importance. Determination of its concentration in milk is commonly applied to monitor the extent to which undesirable changes in flavor, color, and nutritive value of the milk have occurred due to heat treatment.

To determine the desirable heating intensity, the initial concentration of substances in the milk should also be known. Natural substances in milk, including enzymes, often do not greatly vary in concentration, so that a fixed concentration may be assumed. But the content of bacteria or of enzymes excreted by them may vary by several orders of magnitude.

The above-mentioned relations may not be very precise. Possible causes are as follows:

(1) A certain change observed is not the result of one reaction but of several, e.g., consecutive reactions. This is often the case in a complicated material such as milk. If under all conditions considered the same reaction is the rate-determining one the relations may hold, but otherwise they may not. A good example is the heat inactivation of the milk enzyme plasmin, as shown in Figure 6.9B. This plot is markedly curved, which is related to the fact that the protein

molecule should first be unfolded before a second reaction can cause irreversible inactivation. The unfolding of the peptide chain (denaturation proper) is a strongly temperature-dependent reaction, whereas the second reaction generally has a far smaller Q_{10}. In other words, at low heating temperature the former reaction will be the rate-determining one; at high temperature the second reaction, if this reaction is relatively slow. Such relations as well as more intricate relations, often occur.

A complicating factor may be that the different reactions are of different order. As an example, the insolubilization of serum protein by heat treatment is shown in Figure 6.9E. Before the protein is rendered insoluble it should first be denatured, and it subsequently (possibly after other changes in the protein) can aggregate. The aggregation reaction usually follows second-order kinetics, hence the nonlinear relation. This also implies that the curves given in Figure 6.9E do not apply to other protein concentrations.

(2) Apart from what has been mentioned in item 1, even for a single reaction the activation enthalpy (and also the activation entropy; see above) may not be constant. For instance, they depend on the pressure, although this may vary little in the heating processes considered here. There is no general reason why activation enthalpy and entropy should be independent of temperature. In actual practice, however, such independence is usually observed if the temperature range considered is not too wide, though there are exceptions. Especially for protein denaturation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ depend rather closely on temperature. It is also of importance that conditions can alter during the heat treatment. For example, at high temperature acid will gradually be produced in milk, thereby lowering the pH (Fig. 6.1). Usually, the redox potential will also be reduced (Section 1.2.3), partly depending on whether or not the oxygen can be removed during the heating process. Whether there is an effect of all of these change and to what extent will depend on the type of reaction involved. Table 6.4 includes some examples of the influence of pH on the killing of bacteria during heating.

(3) According to Equation 6.7, log K should be plotted against $1/T$ to obtain a straight line. Since t' is inversely proportional to K, we can also use log t'. But plotting log t' against T (or the temperature in °C), instead of against $1/T$, in principle yields a curved plot. An example is in Figure 6.11. If the temperature range considered is comparatively small, a straight line may be acceptable, but extrapolation to other temperatures is usually not allowed. This can readily be seen when the relationship of Z with the temperature is derived:

$$Z = 2.303 \, RT^2/E_a \tag{6.11}$$

Z thus greatly depends on temperature, as is clearly shown in Figure 6.11 where
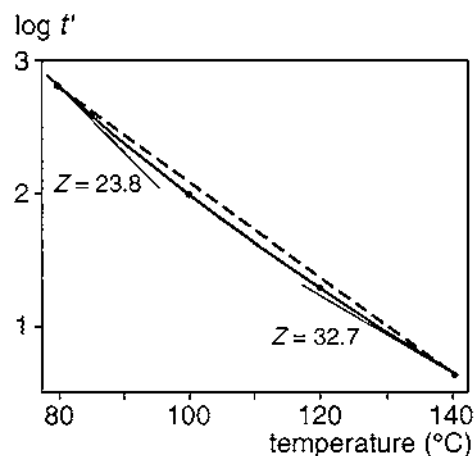
FIGURE 6.11 Example of a relationship between log t' and the temperature as calculated from Equation (6.7) for an activation energy of 100 kJ · mol^{-1}.

Z changes by more than 5% per 10 K in the temperature range considered. We also have

$$Q_{10} \approx \exp\left(10 \, E_a / RT^2\right) \tag{6.12}$$

corresponding to a change of about 5% per 10 K.

(4) In the customary heating processes, the liquid (e.g., milk) is warmed to a temperature of x °C, held at that temperature for y s, and cooled. Assuming that these nominal values are true, in other words that the heating is for y s at x °C, may lead to serious errors.

To begin with, the time for which the product stays in the equipment may vary. In most flow-through processing equipment, this spread is small and often negligible, but it can be fairly large under other conditions. Second, the temperature in the so-called holding section of the heater can markedly decrease, so that the milk can leave the holder at a few degrees lower in temperature than it has entered. This especially occurs in small-scale machinery that suffers significant heat loss by radiation.

Of paramount importance may be the changes occurring during warming and cooling of the liquid. The overall effect of a reaction is given by $\int K(T) \, dt$. When T, and thereby K, are constant, the integral simply yields $t'K(T)$. But if the temperature is a function of time the process is more complicated. Usually, numerical or graphical integration is needed. The result can most readily be described as an effective duration of heating t_{eff}, i.e., the time during which the
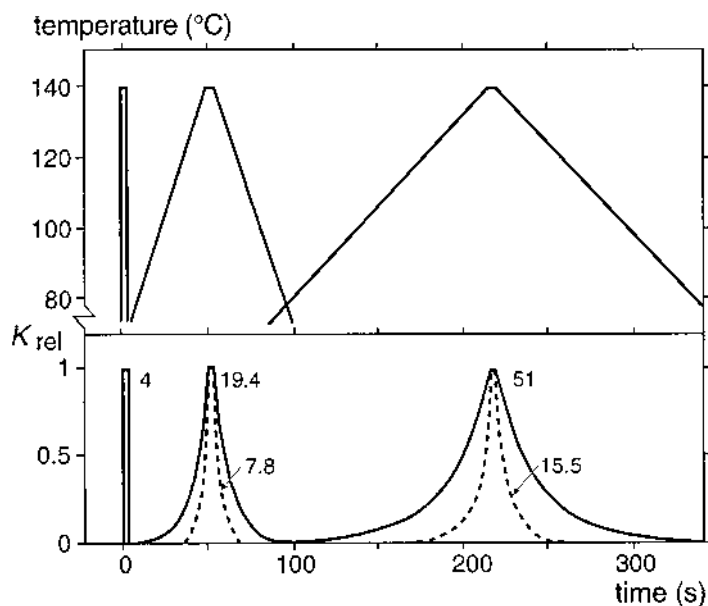
FIGURE 6.12 Schematic examples of temperature profiles during a heating process (nominally 4 s at 140°C) and of the ensuing rate constant compared to that at 140°C (K_{rel}) for an activation energy of 110 (——) and 330 (---) kJ · mol^{-1}. The figures refer to the effective duration of heating (s). The same plot can also be used for other temperatures, e.g., 25–75 instead of 80–140°C (Z then is 21 and 7 K, respectively).

product should be held at the nominal temperature (assuming the times needed for warming and cooling to be negligible) to arrive at the same effect. We thus have

$$t_{eff} = \int_0^\infty K(t)\ dt/K_T \qquad (6.13)$$

where K_T is the rate constant at the nominal temperature. Of course, the result depends on the warming and cooling profiles—hence on the type of apparatus used—but also on the activation energy of the reaction. The smaller the temperature dependence of the reaction, the larger is the difference between the nominal and the effective duration of heating. Figure 6.12 gives some examples of the relative reaction rate as a function of heating time for two warming and cooling profiles and two values of the activation energy. The surface area below the curves represents the effective duration of heating at the nominal temperature. Note that the reaction starts to clearly occur at 120°C for $E_a = 330$ kJ · mol^{-1}

(or $Z = 10$ K at 140°C), which is a common value for the killing of bacterial spores; for $E_a = 110$ ($Z = 30$), typically a value for a ''usual'' chemical reaction, such is already the case at 80°C. Note also that the differences between nominal and effective t' can be considerable.

The subject discussed in item 4 is also of importance in optimizing heating processes. On the one hand, such a change as the killing of spores is desirable, and to achieve this a certain minimum effect is required. On the other hand, changes that cause a decrease in product quality should be largely minimized. The better compromise can be found when all of the particles of milk would precisely undergo the necessary combination of time and temperature. This thus implies that the warming and cooling times should be as brief as possible, the temperature in the holder as constant as possible, and the hold-up time of the milk in the apparatus should vary as little as possible.

(5)   In so-called direct UHT heating (see Section 6.4.2), steam is injected into the milk (or vice versa) to heat it, e.g., from 70°C to 140°C. The steam condenses and the water added is removed again by evaporation at reduced pressure, thereby cooling the milk. During the heat treatment, however, the milk is diluted with water. This would cause bimolecular reactions to proceed more slowly, since the reaction rate is proportional to the product of the concentrations of both reactants. This may apply, for instance, to the Maillard reaction. Reactions causing inactivation of enzymes and killing of microorganisms are probably not affected by the dilution. The heat given up by condensation of the steam would be, for instance, 2.1 kJ per g steam, and the amount of heat taken up by the milk about 280 J per g milk. This implies addition of about 0.13 g of water per g of milk, or a dilution by a factor 0.88. This, then, would mean that a bimolecular reaction would proceed more slowly by a factor 0.88^2, or 0.78.

6.3.3   Thermobacteriology

When the killing of bacteria or their spores is considered, Equation 6.2 is usually written as

$$\log N = \log N_0 - \frac{t}{D} \tag{6.14}$$

where N is colony count. This is illustrated in Figure 6.13, which applies to the simple case of one bacterium species present, and where we will first consider curve a. We can read from the graph that here $D = 2.5$ min. Heating for that time thus would reduce N to 10% of N_0, heating for $2D$ min reduces it to 1%, for $3D$ min to 0.1%, and so on. In curve a, 5.7 min leads to a reduction to 1 organism per ml. Of course, a longer time is needed to achieve the same count if N_0 is higher, e.g., 12.1 min for curve b. At a higher temperature a shorter time is needed, e.g., as for curve c, where $D = 45$ s. If a greater reduction in bacterial
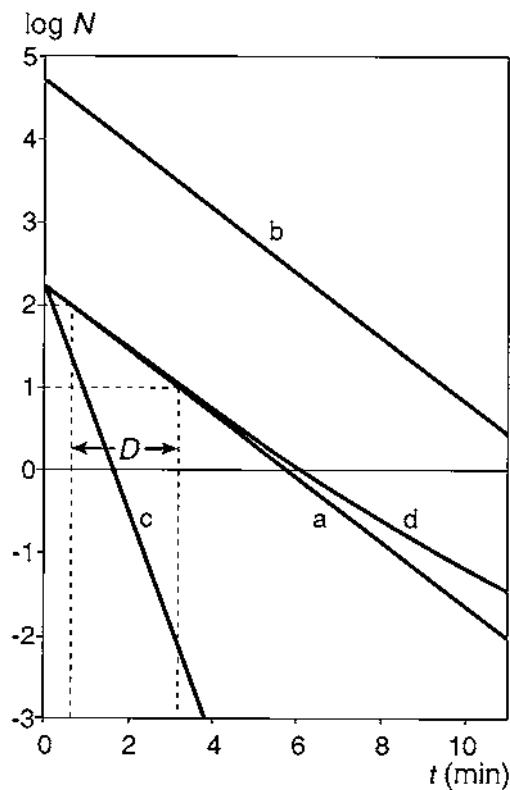
FIGURE 6.13 Examples of the reduction in bacterial count N (in ml^{-1}) as a function of time t during heating of a liquid containing one bacterial species. For curve b the initial count N_0 is higher than for curve a, for curve c the heating temperature is higher; for curve d, see text.

count is desired, e.g., because the initial count is higher, it is generally preferable to increase the heating temperature somewhat, rather than increasing the heating time. This is discussed above in relation to Figure 6.10.

Microorganisms vary greatly in heat resistance. Generally, the characteristic parameters given are D and Z [number of degrees (K) whereby the heating temperature should be raised to reduce D by a factor of 10]. Examples in Table 6.4 show that these parameters vary widely, especially D at a given temperature, but also Z. In modeling sterilization processes, it is sometimes assumed that $Z = 10$ K in all cases for the killing of spores, but that assumption is by no means allowed. It also appears that there can be significant variation within a

TABLE 6.4 Killing of Some Bacteria Due to Heating

	Heating medium	Temp. (°C)	D (min)	Z (K)
Psychrotrophs				
Pseudomonas fragi	Milk	49	7–9	10–12
Pseudomonas fragi	Skim milk	49	8–10	10–12
Pseudomonas fragi	Whey, pH 6.6	49	32	
Pseudomonas fragi	Whey, pH 4.6	49	4–6	10.9
Pseudomonas viscosa	Milk	49	1.5–2.5	4.9–7.9
Pseudomonas viscosa	Whey, pH 6,6	49	3.9	
Pseudomonas viscosa	Whey, pH 4.6	49	0.5	
Pseudomonas fluorescens	Buffer	60	3.2	7.5
Microbacterium thermosphactum	Skim milk	50	2.5	
Listeria monocytogenes	Milk	65	0.1	6.6
Listeria monocytogenes	Skim milk	72	0.07	6.5
Yersinia enterocolitica	Milk	62.8	0.01–0.3	
Other non-spore-forming bacteria				
Salmonella (6 spp.)	Milk	62.8	1.5–4.5	4.0–5.2
Salmonella (2 spp.)	Milk chocolate	62.8	1100–1950	18–19
Staphylococcus aureus	Milk	62.8	7–30	5.0–5.2
Campylobacter jejuni	Milk	50	3.5–5.5	6–8
Escherichia coli	Skim milk	62.8	0.13	4.6
Escherichia coli	Whey, pH 4.6	62.8	0.26	6.7
Streptococcus sp., group D	Skim milk	62.8	2.6	
Streptococcus faecalis	Skim milk	62.8	3.5	
Streptococcus faecium	Skim milk	62.8	10.3	
Streptococcus durans	Skim milk	62.8	7.5	
Streptococcus bovis	Skim milk	62.8	2.6	

Lactococcus lactis ssp. *lactis*	Whey, pH 4.6	62.8	0.32	7.3
Lactococcus lactis ssp. *cremoris*	Whey, pH 4.6	62.8	0.036	6.7
Lactobacillus spp.	Milk	65	0.5–2.0	
Microbacterium flavum	Skim milk	65	2.0	
Microbacterium lacticum	Skim milk	70	4.0	
Spore-forming bacteria				
Bacillus cereus, spores	Milk	121	0.04	9.4–9.7
Bacillus cereus, vegetative	Water or 2 M sucrose	70	0.013–0.016	
Bacillus cereus, germinating spore	Water	70	0.35	
Bacillus cereus, germinating spore	2 M sucrose	70	39	
Bacillus licheniformis	Skim milk, pH 6.7	111	0.48	8
Bacillus licheniformis	Skim milk, pH 6.3	111	0.35	8
Bacillus subtilis, spore	Milk	121	0.03–0.5	10.7
Bacillus subtilis, vegetative	Water	55	1.0–5.6	
Bacillus subtilis, vegetative	2 M sucrose	55	0.8–62	
Bacillus coagulans, spore	Milk	121	0.6–4	
Bacillus pumilus, spore	Milk	95	1.4	9.7
Bacillus stearothermophilus, spore	Milk	121	4–7	8–11
Clostridium sporogenes, spore	Milk, pH 7.0	121	1.7	
Clostridium botulinum, spore	Milk, pH 7.0	121	0.2	
Clostridium botulinum, type A, spore	Phosphate buffer, pH 7.0	110	0.6–0.7	7–12
Clostridium botulinum, type B, spore	Phosphate buffer, pH 7.0	110	0.4–1.1	7–8
Other microorganisms				
Aspergillus sp., conidia	Buffer, pH 4.5	55	2	3.5–4
Aspergillus sp., ascospores	Buffer, pH 4.5	75	2	6–8
Saccharomyces cerevisiae, vegetative	Buffer	60	1	5.0
Saccharomyces cerevisiae, ascospores	Buffer	60	10	5.0
Foot-and-mouth disease virus	Milk	63	~0.2	~10–12

TABLE 6.5   Influence of the Dry Matter Content (Skim Milk, Evaporated Skim Milk, and Skim Milk Powder) on the Killing of Some Bacteria as Caused by Heating

Bacterium	Temp. (°C)	Dry matter (%)	D (s)	Z (K)
Staphylococcus spp.	70	9	70	5.2
	70	93	1800	11.6
Serratia marcescens	50	9	56	4.0
	50	93	1090	13.0
Escherichia coli	63	10	8	4.6
	63	20	15	4.9
	63	30	75	6.3
	63	40	200	7.9

species. In other words, different strains of one species can have different heat resistances. Furthermore, the conditions during heating can affect D and Z. For example, compare in Table 6.4 milk and whey, or the same liquids at different pH values. Small changes in composition of the heating medium may have a considerable effect, but the explanation is uncertain. The heat resistance of microorganisms often increases with the dry matter content of the medium, and the temperature dependence may decrease. Examples are given in Table 6.5.

   Often deviations from the ideal reaction kinetics for the killing of bacteria and spores occur, and we first consider the items mentioned in Section 6.3.2. Generally, deviation from simple first-order kinetics is small (item 1). Also, item 2 does not greatly affect kinetics because it generally concerns a small temperature range. The reaction involved may, however, change in rate because of changes in the milk during heating. For example, for some species in Table 6.4 the reaction rate may be shown to increase by about 10% as a result of a decrease of the pH of the medium by 0.1 unit. The changing redox potential of the medium can also affect the reactions occurring during the killing, as some bacteria are more heat-sensitive at a higher redox potential. The main deviation, however, stems from item 4. It implies that for every individual apparatus involved the effective duration of heating as a function of Z should be calculated.

   Moreover, in the killing of bacteria and spores some specific deviations may occur. Thus the following can be stated:

   (1)   The heat resistance of organisms varies, even within a strain. This is no surprise, considering that it concerns living organisms that show genetic variation. Apart from that, individual cells may have grown under different conditions. On average, the most heat-sensitive cells will be killed first during heating, so that the remaining population increases in heat resistance. In other words, K de-

creases and D increases during heat treatment. Plotting log N against time will not yield a straight line throughout but a slightly upcurved one (see Fig. 6.13, curve d). The deviation may be stronger if it concerns a mixture of different strains. Naturally, strongly curved relations are observed for a mixture of bacteria, such as the population of organisms in most raw milk.

(2) Heating the medium for a short time is sometimes found to increase the colony count. It is important to note that colony count is defined as the number of colony-forming units (CFU) per ml. Because of the turbulence in the heater, organisms that were present in aggregates can disintegrate into separate cells and hence yield a higher count.

(3) A heat treatment that kills the vegetative cells of an organism can keep its spores alive and can even activate the germination of the latter. For example, milk contaminated by *Bacillus cereus* and subsequently heated for 30 min at 70°C will spoil due to growth of the organism. All vegetative organisms then are killed, but not the spores (see Table 6.4). Cooling the milk after the heating, immediately followed by heating and cooling it again, causes the count of *B. cereus* (including spores) to diminish by 1–2 decades. Moreover, the subsequent growth appears slower. In other words, the former heating causes germination of spores and the second one kills these cells.

(4) The properties of the milk as a growth medium can have been altered due to the heat treatment. This is illustrated in Figure 6.14, which shows that there are large differences. Of course, the changes in properties are of major importance with reference to the spoilage of heated milk or to the growth of bacteria in fermented products. They can also affect the apparent count after heating (as determined by establishing the minimum volume of a milk sample in which growth occurs). In principle, the following are the changes caused by heating the milk.

    a. *Inactivation of inhibitors. (1) Immunoglobulins*. The combinations of time and temperature for inactivation of this class of inhibitors largely coincide with those for the inactivation of cold agglutination (Fig. 6.9D). *Bacillus cereus* is fairly sensitive to immunoglobulins because IgM (Section 2.4.3) causes the bacterial cells to agglutinate and to sediment to the bottom of the vat. *(2) Lactoperoxidase system*. Most of the lactic acid bacteria are fairly sensitive; most gram-negative bacteria are not. The system is inactivated by denaturation of the enzyme, but its activity should be reduced to at least 0.001 because peroxidase is in excess in milk. The effects of inactivations (1) and (2) are seen in Figure 6.14 at about 60°C and 70°C, respectively. (3) *Bacillus stearothermophilus* is sensitive to *lactoferrin* (the bacterium grows faster in milk when ferro salts have been added), which appears to be inactivated as a bacterial inhibitor at a much more intense heating than is needed
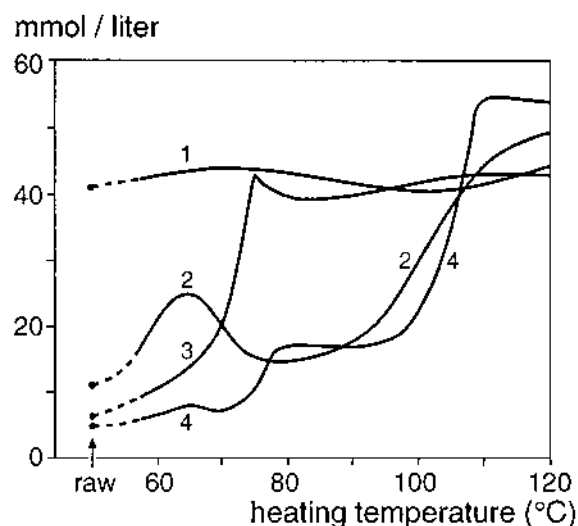
mmol / liter

FIGURE 6.14   Acid production in milk, after inoculating it with 0.01% bacterial culture and keeping for 18 h. The milk had been heated at various temperatures for 20 min before inoculation. The cultures involved were strains of the following organisms: *Lactococcus lactis* ssp. *lactis* (curves 1 and 3, incubation temperature 30°C); *L. lactis* ssp. *cremoris* (3, 30°C); *Streptococcus thermophilus* (2, 42°C); *Lactobacillus lactis* (4, 42°C). After results by J. Auclair and A. Portmann, *Ann. Technol. Agric.* (INRA) **7** (1958) 129.

for its denaturation. Not surprisingly, *B. stearothermophilus* hardly grows, if at all, in UHT milk, but it does grow in traditionally sterilized milk. The effect of lactoferrin can considerably affect the analysis of the kinetics of the killing of bacteria.

b. *Formation of stimulants*. Some lactic acid bacteria, especially the thermophilic ones, are enhanced by the presence of formic acid, which is formed during intense heating (Section 6.2.3). This may explain the stronger acid production (more rapid growth) at heating temperatures above 100°C in Figure 6.14, curves 2 and 4.

c. *Formation of inhibitors or inactivation of stimulants*. For example, *Bacillus stearothermophilus* has been found to grow more slowly in moderately heated milk than in raw milk.

d. *Killing of bacteriophages*. This will rarely play a role because raw milk contains at most a very low number of phages (e.g., 1 or less per liter) for the organisms present.

In sterilizing food products, the bactericidal action is expressed as the sterilizing effect S, i.e., the number of decimal reductions:

$$S \equiv \log N_0 - \log N \tag{6.15}$$

When it concerns one bacterium species (or essentially its spores), S can be calculated from Equation 6.15 if the effective duration of heating is known. The desirable sterilizing effect will be selected depending on the estimated initial count N_0. Obviously, in a sterilized product the risk of spoiling should be very slight, e.g., $< 10^{-5}$. For example, for bottles containing 1 L of milk this implies that after sterilization $N < 10^{-8}$ (cells per ml). Assuming N_0 to be up to 10^3 then necessitates $S = 11$. Because of the above-mentioned uncertainties a safety margin is usually taken into account. Checking the heating process is difficult because in actual practice it is impossible to determine a colony count that is as low as 10^{-8}. Furthermore, the effective temperature and duration of heating of a sterilization process often are hard to determine. A suitable heating intensity may be determined by preparing pilot experimental batches of the milk that are heated at the sterilizing temperature following addition of a known large number of the spores involved, e.g., 10^7 per ml; now $S = 11$ means that on keeping the milk, 9 of every 10 liter bottles should keep sterile, and this can indeed readily be determined. However, this test is not quite correct, first because of the above-mentioned deviation of the linearity of the plot of log N against t (see Figure 6.13, curve d), and second because in actual practice more strongly heat-resistant strains can occur than those used in the test. Consequently, a certain margin remains necessary.

6.3.4 Inactivation of Enzymes

Heat inactivation of most enzymes follows first-order kinetics as occurs during denaturation of globular proteins. The inactivation is strongly temperature-dependent, Q_{10} mostly being at least 50. A D value of 1 min is usually reached between 60°C and 90°C. But milk contains some enzymes that can cause spoilage and that show a strongly differing heat inactivation (Table 6.6).

After the actual denaturation of the enzyme molecule, at least one ensuing reaction is needed to prevent renaturation of the enzyme from occurring on cooling. (Renaturation would mean preserving the enzyme activity.) Various enzymes are inactivated at far higher heating intensities than mentioned above, and they also show a lower Q_{10}; see, for instance, Figure 6.9B. The high heating intensity then is needed for the ensuing reactions to proceed because these enzymes generally are in a denatured state at a temperature of, say, 80°C. The nonlinear relationship between log t' and temperature that then often occurs is discussed in Section 6.3.2, item 1.

TABLE **6.6**   Heat Inactivation of Some Enzymes in Milk

Enzyme	EC number	Temp. (°C)	D (s)	Q_{10}
Milk enzymes				
Alkaline phosphatase	3.1.3.1	70	33	60
Lipoprotein lipase	3.1.1.34	70	20	13
Xanthine oxidase	1.1.3.22	80	17	46
Lactoperoxidase	1.11.1.7	80	4	230
Superoxide dismutase	1.15.1.1	80	345	150
Catalase	1.11.1.6	80	2	180
Plasmin	3.4.21.7	80	360	3.3
Plasmin	3.4.21.7	120	30	(1.5)
Acid phosphatase	3.1.3.2	100	45	10.5
Extracellular bacterial enzymes[a]				
Lipase *Pseudomonas fluorescens*		130	500	(1.3)
Lipase *Pseudomonas* sp.		130	700	2.4
Lipase *Alcaligenes viscolactis*		70	30	2.6
Proteinase *Pseudomonas fluorescens*		130	630	2.1
Proteinase *Pseudomonas* sp.		130	160	1.9
Proteinase *Achromobacter* sp.		130	510	2.1
Chymosin	3.4.23.4	60	25	70

[a] The results may vary widely among strains and may also depend on conditions during growth.

Still other deviations may occur. Figure 6.15A gives an example of so-called low-temperature inactivation. The protease molecules ''consume'' each other, i.e., at a temperature at which part of the molecules is in a denatured state, the molecules in the native state can proteolytically hydrolyze the former, thereby inactivating them. Most native globular proteins are quite resistant to proteolysis. The denaturation itself appears to occur at fairly low temperature and to have a high Q_{10}; that does not hold true for the heat inactivation (which in this case proceeds roughly as in Figure 6.15C). Naturally, the protease can also hydrolyze other proteins if these are in an unfolded state. This would be the explanation for the unexpected shape of the curves in Figure 6.15B, i.e., at fairly low temperature native undenatured protease can attack the lipase that is already denatured. In actual practice the extent to which low-temperature inactivation occurs will therefore greatly depend on the warming and cooling rates applied.

The relationship is again different in Figure 6.15C. Probably, three ensuing reactions occur, the first two being reversible:
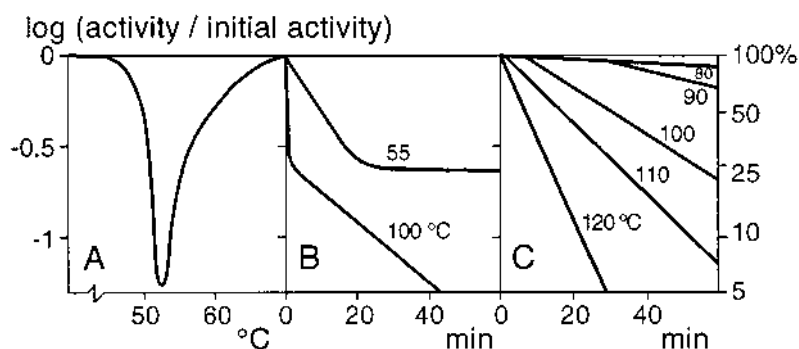
FIGURE 6.15 Heat inactivation (expressed as residual activity) of some bacterial enzymes in milk. (A) Protease of a *Pseudomonas fluorescens*; heating for 30 min at the temperature indicated. (B) Lipase of the same bacterium during heating at two temperatures. (C) Protease of an *Achromobacter* sp. during heating at various temperatures.

1. A $\rightleftarrows$ B rate constants K_1, K_{-1}
2. B $\rightleftarrows$ C rate constants K_2, K_{-2}
3. C $\rightarrow$ D rate constant K_3

where A is the native state of the enzyme molecule, B an unfolded state, and C an intermediate. Only the D state is irreversibly changed. K_1 is typical of denaturation, K_2 becomes essential above 80°C, and K_3 above about 95°C. All this refers to Figure 6.15C. K_{-1} often is fairly large. The magnitude of K_{-2} is of little importance, unless it is very small. In the latter case, the enzyme may exhibit a slow reactivation after cooling. Alkaline phosphatase as well as lactoperoxidase can show a slight reactivation after the heated milk has been kept cool for several days.

Finally, when plotting log activity against time, a straight line is not always obtained, probably because the enzyme occurs in two or more forms, mostly genetic variants. These isozymes may show different inactivation kinetics.

Some enzymes will now be briefly discussed.

Lipoprotein lipase (EC 3.1.1.34) of milk is somewhat deviating from the norm because the Q_{10} for heat inactivation is fairly small, i.e., about 10 at 75°C ($Z \approx 10$ K).

Plasmin (EC 3.4.21.7) is very heat-resistant, as Figure 6.9B shows. Above 110°C, the inactivation rate increases only slightly with increasing temperature. Even at 140°C, the milk should be heated for at least 15 s to prevent the occur-

rence of proteolysis during keeping. To be sure, the plot is more or less uncertain, since the bulk of the enzyme occurs in milk as an inactive zymogen, the plasminogen, which can be slowly transformed into the active form by the enzyme urokinase (Section 2.5.2.5). Plasminogen also is very heat-resistant.

Bacterial lipases, especially lipases excreted in the milk by some gram-negative rods, may be very heat-resistant (Table 6.6).

Bacterial proteinases, especially extracellular endoproteinases of gram-negative rods, can also be very heat-resistant (Table 6.6). Often, one inactivation reaction with a small Q_{10} (roughly 2) is found, but in other cases two reactions can be distinguished (see Fig. 6.15C).

As a consequence of the incomplete inactivation of lipases, lipolysis may cause a rancid flavor. Residual milk proteinase especially attacks β- and α_{s2}-caseins. As a result, a bitter flavor may develop, and skim milk may finally become more or less transparent. Residual bacterial proteinases mainly attack κ-casein. Consequences may be bitter flavor development, gel formation, and wheying off.

The only measure against the action of the milk enzymes is an adequate heat treatment. Most of the bacterial enzymes mentioned are insufficiently inactivated by heat treatment because of their great heat resistance. Therefore, the only corrective measure is to prevent growth of the bacteria involved.

6.4   METHODS OF HEATING

Heating (and cooling; see Chapter 10) of liquids can be done in several different ways, and with various kinds of machinery.

6.4.1   Considerations

Prerequisites for a heating process may be defined as follows:

    a.   The desirable time–temperature relationship should be practicable. It also involves such aspects as controllability and reliability, and uniformity of heating. In establishing the result of the heat treatment (e.g., the sterilizing effect), the times needed for warming and cooling should be accounted for (see Section 6.3.2).

    b.   No undesirable changes should occur in the product, such as absorption of extraneous matter (including Cu, Sn, plasticizers), loss of compounds (e.g., water), disruption or coalescence of fat globules, coagulation of protein, etc. Sometimes, excessive growth of thermophilic bacteria can occur in a pasteurizer.

    c.   The expenses should be low. They partly depend on the price, the lifetime, and the maintenance and operating costs of the machinery. Of much concern is the amount of energy needed for heating and cooling,

which may be kept low by regeneration of heat and cold, respectively. Furthermore, the extent of fouling plays a role. Rapid fouling causes the heat transfer and the rate of flow to diminish. As a result, consumption of energy increases significantly. This necessitates frequent cleaning, hence brief operating times.

d. The way of working should fit into the planning. For example, insertion of operations like centrifugation or homogenization in the process line may be desirable and so may be good possibilities to adjust heating temperature, heating time, and flow capacity.

Furthermore, a particular apparatus or processing scheme is selected on the basis of:

a. The desirable combination of time and temperature. Heating for 30 min at 68°C requires other machinery than 1 s at 145°C.

b. Properties of the liquid. The main factor involved is the heat transfer rate which, in turn, depends on the thermal conductivity and especially on the viscosity (see Section 6.5). Apart from that, the tendency to exhibit fouling is of importance (see Section 12.1). Generally, highly viscous products show poor heat exchange, as is illustrated in Table 6.9, further on.

c. Requirements for the prevention of recontamination and for ensuing process steps, especially packing.

An additional factor in the selection may be the effect of the method of heating on the air content, especially the O_2 content of the milk. After all, the O_2 content affects the possibilities for growth of several bacteria. For example, *Bacillus* types need some O_2; lactic acid bacteria are slowed down at high O_2 pressure. The O_2 content of long-life milk products may affect the development of off-flavor by fat autoxidation. Holder pasteurization causes significant deaeration, but air can be reabsorbed during cooling. Heating in a heat exchanger does not affect the O_2 content, unless a special deaerator (e.g., a flash cooler) is connected, as in direct UHT treatment (Section 6.4.2). The extent of deaeration during autoclaving depends on the type of sealing applied. For example, bottles fitted with crown corks lose most of the air, but sealed cans lose nothing.

6.4.2 Equipment

Liquids can be heated and cooled in a batch process, in a heat exchanger, or in a packed form. Originally, batch processing was in general use for pasteurizing beverage milk. It is the so-called *holder pasteurization*, e.g., 30 min at 63°C. The method is still in use in the manufacture of starters, whipping cream, and other small-scale products. Usually, the jacketed vats involved are fitted with an agitator; through the double jacket, steam or hot water circulate followed by
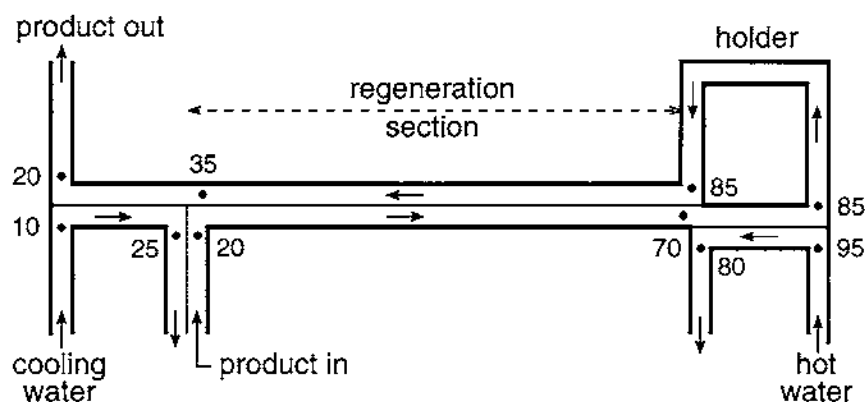
FIGURE 6.16 Simplified scheme of a heat exchanger for heating and cooling of liquids, showing the principle of regeneration. The numbers are temperatures (°C) and merely give an example. Thick lines denote insulating walls; thin lines are walls allowing rapid heat transfer.

cold water. Among the advantages of holder pasteurization are the simplicity, flexibility, and satisfactory temperature control (little fluctuation in temperature unless highly viscous liquids are used). A drawback is that the warming and cooling times are long (excessive for large containers). Furthermore, regeneration of heat is not well possible and connection to continuous processes is awkward.

Currently, flow-through heaters or heat exchangers are commonly used. Hot water or condensing steam constitute the heating medium. Sometimes, vacuum steam heating is applied to minimize the difference in temperature with the liquid to be heated.

In *plate heat exchangers*, a large heating surface is assembled in a confined space and on a small floor area. Heating agent and incoming liquid are present in thin layers and are separated by a thin wall, i.e., a plate. Because of the large heating surface per unit volume of liquid that is to be heated, the difference between the temperature of the heating agent and the temperature of the liquid to be heated can be small, e.g., 2°C when milk is heated from 65°C to 75°C. This may be an advantage for some heat-sensitive products, where fouling of the heat exchanger is greater for a higher wall temperature. Furthermore, warming and cooling proceed rapidly in plate heaters.

Another advantage is that the energy consumption (for heating and cooling) can be relatively small because heat can be regenerated. The principle is shown in Figure 6.16. When the milk comes in the heat exchanger, it is heated by milk that has already been heated and that is at the same time being cooled by the
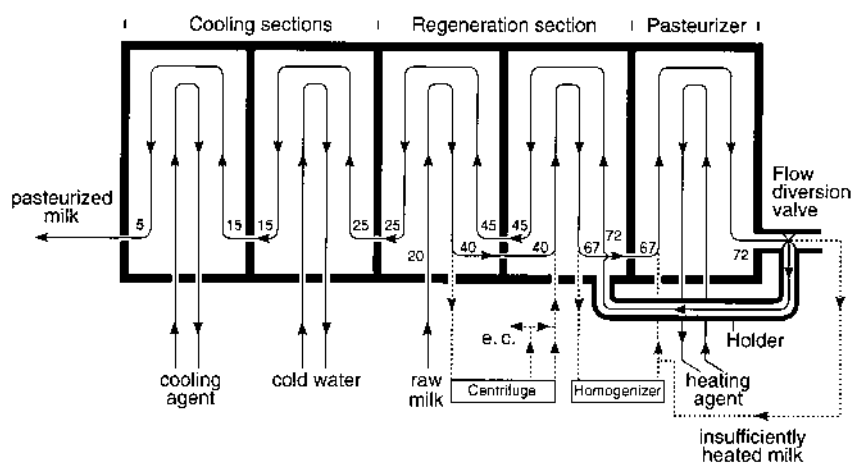
FIGURE 6.17 Example of a pasteurization process in a plate heat exchanger. Simplified diagram. e.c., excess cream; temperatures in °C.

milk coming in. The latter is subsequently being heated further by hot water (or steam). It may then flow through a holder section, to achieve a sufficient heating time. After being cooled by the incoming milk, it is cooled further by means of cold water (or another cooling agent). Note that the liquids being heated and cooled are always in counterflow and that the temperature difference between the two remains constant (see Section 6.4.3).

A drawback of plate apparatus is their vulnerability. The rubber gaskets can become leaky, they do not resist high pressures, and they should occasionally be changed. Cracks can be formed in the plates. As a result, small amounts of raw milk might leak into the milk already pasteurized, e.g., in the regeneration section, possibly with detrimental results on bacterial quality. Furthermore, plate heaters are only fit for heating liquid products. To attain a high enough speed through the machinery, highly viscous liquids require such a high operating pressure that leakage may occur. Moreover, heating to above 100°C requires special construction because it involves pressures over 1 bar (see Figure 6.21).

A plate heat exchanger is made up of various sections connected in series, including regeneration section, heating section, holding section (may also be a tube), and cooling sections. Each section consists of a great number of plates, being partly parallel and partly connected in series. In this way, the liquid is properly distributed among the plates and arrives at a high enough speed to reduce fouling. Figure 6.17 gives an example of the constructional setup of a plate heat exchanger and of the path of the liquids through it. The plates are shaped in such
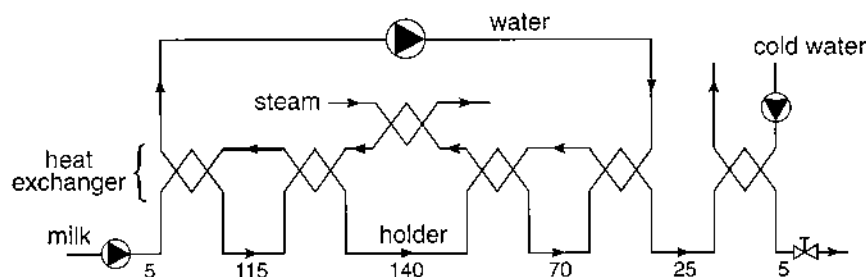
FIGURE 6.18 Example of the flow diagram of a heat exchanger in which the milk is heated and cooled by water only. The figures refer to the milk temperature in °C.

a way as to greatly enhance turbulence in the liquid. This enhances heat transfer and diminishes fouling.

Tubular heat exchangers generally have a smaller heating surface per unit volume of liquid to be heated than plate heat exchangers. Accordingly, the difference in temperature between the heating agent and the incoming liquid is greater. To restrain fouling and enhance heat transfer, high flow rates are used, which necessitates high pressures. But this causes no problems because tubes are much stronger than plates; after all, tubular heat exchangers have no sealing gaskets but mostly have (spirally bent) concentric tubes. Tubular heat exchangers can readily be applied to obtain very high temperatures (e.g., 150°C). Accordingly, they are excellently fit for indirect UHT treatment. Like a plate heat exchanger, a tubular heat exchanger can be built of regeneration, heating, holding, and cooling sections.

In modern heat exchangers, the milk may be in counterflow with water throughout the apparatus. The water is kept circulating and is heated by means of indirect steam heating, immediately before it should heat the milk to the maximum temperature desired. Figure 6.18 shows an example. Often, up to about 90% heat regeneration is achieved. This way of working has advantages with reference to temperature control, rapid and even heat transfer, and energy saving.

In *UHT treatment with direct heating* there is no wall between the heating agent and the liquid to be heated, but the heating agent (steam) is injected into the liquid or the other way around. Thereby an almost instantaneous heating to the desired temperature (e.g., in 0.1 s from 80°C to 145°C) occurs, provided that the steam can immediately condense. For this to happen, finely dispersed steam and a significant back pressure in the liquid are needed (see Fig. 6.21). The incoming liquid becomes diluted with the condensing steam. Of course, the steam has to be of high purity. After maintaining the liquid at the desirable temperature
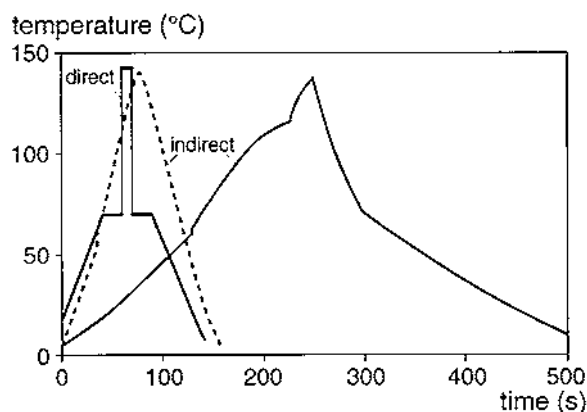
FIGURE 6.19 Temperature of milk versus time during heat treatment. One example refers to direct and two examples to indirect UHT heating.

for a few seconds, it is discharged in a vessel of reduced pressure. Here almost instantaneous evaporation of water occurs, causing very rapid cooling. The amount of water evaporating should equal the amount of steam having been absorbed before.

Steam injection heating causes disruption of fat globules and some coagulation of protein. Homogenization (aseptic) at high pressure redisperses the coagulum. Without homogenization, the product gives an inhomogeneous astringent or nonsmooth sensation in the mouth, and a sediment tends to form on storage.

The heating section (up to, e.g., 80°C) that precedes the steam injection, and the cooling section connected after the ''flash cooling'' (starting from, e.g., 80°C), may be plate heat exchangers. Figure 6.19 shows that the holdup time of the milk above, say, 80°C is very short, i.e., insufficient to inactivate milk proteinase. Therefore, holding times are often extended or the warming that precedes the steam injection is made slower and extended to a higher temperature. Also in indirect UHT treatment, long warming times are sometimes applied to obtain a fuller inactivation of milk proteinase. In this way, the difference between traditionally sterilized and UHT milk is diminished.

The so-called vacreator is quite similar to a direct UHT heater, but in this apparatus the liquid is heated to pasteurization temperatures. The essential detail is evaporative cooling in vacuum, aimed at removal of volatile flavor components. Such apparatus is occasionally used for pasteurizing cream for butter manufacture. Disadvantages are the considerable damage (coalescence and disruption) to fat globules, and the limited heat regeneration.

Autoclaving. Heating a liquid in a hermetically sealed container (rarely

larger than 1 L) has the distinct advantage that recontamination of the heated liquid by microorganisms can be readily prevented. That is the reason why this method of working is primarily applied in sterilization. After all, it suffers from great disadvantages. The long warming and cooling times, and the large temperature differences inside a can or bottle, result in undesirable changes like browning and sterilized milk flavor. Agitating or revolving the containers during the heat treatment may restrict these disadvantages since it considerably enhances heat transfer and evenness of temperature. However, its technical operation is difficult because the heating by steam must occur under pressure, at least when sterilization is applied. Heating to 120°C corresponds to a gauge pressure of 1 bar (see Fig. 6.21).

The easiest way of heating is batchwise in a steam closet or autoclave. But it implies low-capacity, high-energy costs (regeneration is impossible) and is very laborious. Because of this, continuous sterilizers are commonly applied. For bottles, it mostly concerns a hydrostatic sterilizer in which the bottles pass twice through a water seal, about 10 m high because of the 1-bar gauge pressure (Fig. 6.20). For cans, the so-called ''cooker and cooler'' with rotary air locks is usually used. Continuous sterilizers allow considerable heat regeneration.

6.4.3   Heat Regeneration

Regeneration is the regaining of heat and the saving of cooling energy in the combined processes of heating and cooling (see Fig. 6.16). The regenerating effect is the heat absorbed in the regeneration section as a percentage of the total heat absorption. If the specific heat is the same for all liquids at all temperatures, the regenerating effect can simply be calculated from:

$$\frac{\text{Temperature after heating up in regeneration section} - \text{inlet temperature}}{\text{Pasteurization or sterilization temperature} - \text{inlet temperature}} \times 100\%$$

Theoretically, as much as 100% regeneration might be achieved, but this would need an infinite heating surface and absence of heat loss to the surroundings. Loss of heat does, however, occur, and the surface area is by no means infinite. There is an optimum at which the additional savings on heating and cooling energy and the debit on additional plates, etc. balance each other. It often amounts to about 90% regeneration.

A high proportion of regeneration also has disadvantages. To begin with, it takes a long time for the heat exchanger to arrive at the desired temperature, up to 1 h. This not only plays a part during startup of the heating process (which is done with water), but also in cleaning of the exchanger. (It then might become tempting to make shift with cleaning at a lower temperature!) Therefore, a special
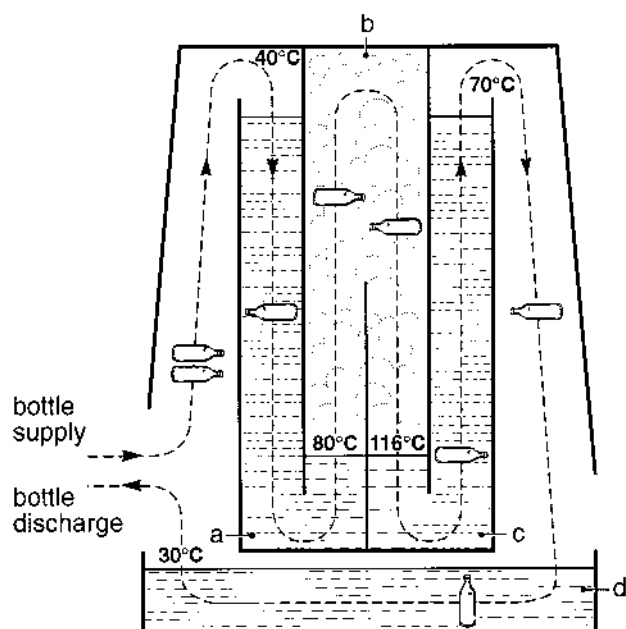
FIGURE 6.20 Diagram of a continuous in-bottle sterilizer. a, Water seal in the bottle entrance and preheating section; b, steam space with adjustable pressure; c, water seal in the bottle discharge and first cooling section; d, second cooling section. Indicated temperatures refer to that in the bottles. The water is partly circulated.

heat exchanger for heating up of the heating plant is sometimes connected. A second difficulty is that the heating up of the milk tends to last a long time. In the simplest case, such as shown in Figure 6.17, the temperature difference in the regeneration section between the milk that is warming and the milk that is cooling down, ΔT, is constant; it is equal to the difference between pasteurization temperature and temperature after regeneration. A greater heat regeneration then leads to a smaller ΔT. ΔT is proportional to $(1 - r)$ if r is the fraction of heat regenerated. At a smaller ΔT, the heating surface should be larger and hence the holdup time in the regeneration section longer. Clearly, the warming time of the milk is inversely proportional to $(1 - r)$. Consequently, increasing the regenerating effect from 70% to 90% results in a threefold increase in warming time. Figure 6.19 gives an example of a very long warming time, intentionally selected to regenerate much heat. Comparing this example with Figure 6.12 (which has

roughly the same hypothetical course of the temperature) shows the strong effect of a great heat regeneration on the effective heating time, especially for reactions of a low Q_{10}.

6.4.4   Control

It goes without saying that the heated product must be safe and of satisfactory quality, and this necessitates rigorous control. That may be achieved by control of the properties of the final products. But provisions against risks should also be made during heat treatment. The following are the main risks:

a.   The heating intensity may be insufficient because steam supply, hence heating temperature, may fluctuate or because of a sudden increase in fouling. Often, a pasteurizing plant has a so-called automatic flow diversion valve. The milk flows back to the supply pipe if the pasteurization temperature decreases to below a preset limiting value (see also Fig. 6.17). Alternatively, an automatic ''pump stop'' may be applied. Moreover, the heating temperature should be recorded continuously. The risk of too brief a heating time will be slight: the volumes in the heat exchanger (especially the holder) are fixed, and it is almost impossible that the milk pump would suddenly run faster.

b.   Recontamination is a factor. Raw or insufficiently heat-treated milk may gain entrance into the heated milk, e.g., because of a leaky heat exchanger or because a mistake is made in connecting pipes. Naturally, contamination occurs when milk passes through a machine or pipe that is not absolutely clean. Recontamination should be rigorously avoided in UHT treatment, because it is usually combined with aseptic packing (Chapter 13); it specifically concerns the homogenizer. One bacterium per 1000 L of milk may cause unacceptable spoilage. However, in pasteurized and thermalized milks relatively small recontaminations can also have considerable effects.

c.   Growth of bacteria may occur in heating equipment, e.g., in a batch pasteurizer. Especially in a vessel such as a balance tank through which milk flows while it maintains a relatively high temperature for some time, organisms like *Bacillus stearothermophilus* (maximum growth temperature ranging from 65°C to 75°C) and *B. coagulans* (maximum 55°C to 60°C) can grow. As a rule, the counts of these bacteria in raw milk are very low. Accordingly, the contamination will become perceptible only after many hours. Obviously, occasional cleaning and disinfection of the machinery can overcome these problems.

   After having been in use for hours, a pasteurizer may contain growing bacteria in that part of the regeneration section where the milk is being cooled. The bacteria involved survive the milk pasteurization

and may colonize on the metal surface of plates or tubes that show some fouling by milk components; such a layer of bacteria and milk components is called a biofilm. Bacteria in a biofilm can grow rapidly, so that significantly increased counts in the pasteurized milk may be found after, say, 10 h of continuous use of the apparatus. It mostly concerns *Streptococcus thermophilus* (maximum growth temperature about 53°C). But *S. faecium* (= *S. durans*, maximum ~52°C) and *S. faecalis* (maximum ~47°C) may also cause problems. Timely cleaning is the obvious remedy.

6.5 APPENDIX: HEAT TRANSFER

Some aspects on the subject of heat transfer are briefly summarized here. Numerical data are given in Tables 6.7 to 6.9 and in Figure 6.21.

The amount of heat that has to be transferred per unit time for heating a liquid from temperature T_1 to T_2 (without heat of fusion, heat of reaction, etc., occurring) is given by

$$q = (T_2 - T_1) \, Q c_p \, \rho \qquad (6.16)$$

where Q = liquid flow rate ($m^3 \cdot s^{-1}$), c_p = specific heat of the liquid, and ρ =

TABLE 6.7 Approximate Examples of the Effect of Composition and Temperature of a Milk Product on the Coefficient of Heat Conductivity λ ($W \cdot m^{-1} \cdot K^{-1}$), the Viscosity η (mPa $\cdot$ s), and the Specific Heat c_p ($kJ \cdot kg^{-1} \cdot K^{-1}$) (Any Heat of Fusion Not Included)[a]

	0°C			20°C		80°C	
Product/material	λ	η	c_p	λ	η	λ	η
Water	0.57	1.79	4.2	0.60	1.00	0.66	0.36
Skim milk		3.45	3.8	0.54	1.68	0.63	0.56
Whole milk	0.45		3.9	0.52	1.93	0.61	
Concentrated milk 1:1.9			3.5	0.48	3.1	0.56	
Concentrated milk 1:2.5			3.2	0.45	6.3	0.53	
25% fat cream	0.32		3.5	0.37	4.2		
45% fat cream	0.28		3.2	0.32	13.5		
Milk fat	0.13		2.2	0.17	71		
Air				0.02			
Stainless steel				17			
Copper (red brass)				371			

[a] η of concentrated milk closely depends on preheating, η of cream on homogenization, if carried out. For comparison, λ of air and of two metals is given.

TABLE 6.8   Approximate Examples of the Individual
Coefficient of Heat Transfer α of Some Media Onto a Wall,
Under Various Conditions

Medium	Condition	α in $W \cdot m^{-2} \cdot K^{-1}$
Air	Flowing	10–100
Water	Flowing	600–6000
Water	Boiling	2000–7000
Steam	Condensing	6000–17000
Whole milk	~38°C; Re = 10^4	800
	~38°C; Re = 10^5	2900
	~70°C; Re = 10^4	500
	~70°C; Re = 10^5	2000
25% fat cream	~38°C; Re = 10^4	650
	~70°C; Re = 10^4	450

liquid density. For example, heating milk from 10°C to 74°C at a flow rate of
7200 L · h^{-1} consumes about 5×10^5 W (according to data in Table 6.7).

When two liquids are kept separate by a fixed wall, the heat transfer from
one liquid to the other is given by

$$q = A\ \Delta T k_h \tag{6.17.a}$$

where A = surface area of the wall, ΔT = temperature difference between the
liquids, and the total coefficient of heat transfer is given by

$$(1/k_h) = (1/\alpha_1) + (\delta/\lambda) + (1/\alpha_2) \tag{6.17.b}$$

where α = individual coefficient of heat transfer from the wall to the liquid or
vice versa, δ = thickness of the wall, and λ = coefficient of heat conductivity
of the wall material. Assuming k_h to be 1 kW · m^{-2} · K^{-1}, then in our example
a heating surface of 25 m^2 would be required if ΔT is 20 K, and 100 m^2 at 5
K. Obviously, to keep the heating surface as small as possible at a given ΔT,
maximization of the total heat transfer coefficient would be needed.

c_p scarcely depends on temperature, and λ and ρ only a little. But the α
values greatly depend on conditions (see Table 6.8). The media involved (milk
product and heating or cooling agents) considerably affect α, but most of all the
flow does. In laminar flow, α is very small because all heat must be transferred
through the fluids by diffusion. Natural convection can increase α, but above all
turbulent flow can lead to high α values. The higher the Reynolds number (Re),

TABLE **6.9** Coefficient of Total Heat Transfer k_h Under Various Conditions[a]

Heating or cooling agent	Product	Condition	k_h (average) in $W \cdot m^{-2} \cdot K^{-1}$
Surrounding air	Whole milk	Tank with empty double jacket, no stirrer	3
Water	Water	Double-walled tank, stirrer and scraper	640
Water	35.5% fat cream	Same	350
Water	Same, soured	Same	215
Water	35.5% fat cream	Same, but no scraper	230
Water	Yogurt	Same	290
Water	Yogurt	Same, but stirring rate halved	140
Water	Water	Plate heat exchanger, re-generation section	3200
Water	Water	Plate heat exchanger, heat-ing section	4900
Water	Water	Plate heat exchanger, cool-ing section	3500
Steam[b]	Whey	Evaporator[c] 40°C	1400
Steam[b]	Whey	Evaporator[c] 70°C	3300
Steam[b]	Concentrated whey 50% dry matter	Evaporator[c] 40°C	750
Steam[b]	Concentrated whey 50% dry matter	Evaporator[c] 70°C	2300
Steam[b]	Skim milk	Evaporator,[c] first effect	2300–2600
Steam[b]	Skim milk (concen-trated)	Evaporator,[c] second effect	1900–2200
Steam[b]	Skim milk (concen-trated)	Evaporator,[c] third effect	1000–1200
Steam[b]	Whole milk	Evaporator,[c] first effect	2000–2200
Steam[b]	Whole milk (concen-trated)	Evaporator,[c] second effect	1700–1900
Steam[b]	Whole milk (concen-trated)	Evaporator,[c] third effect	900–1100

[a] Approximate results.
[b] Condensing steam or water vapor.
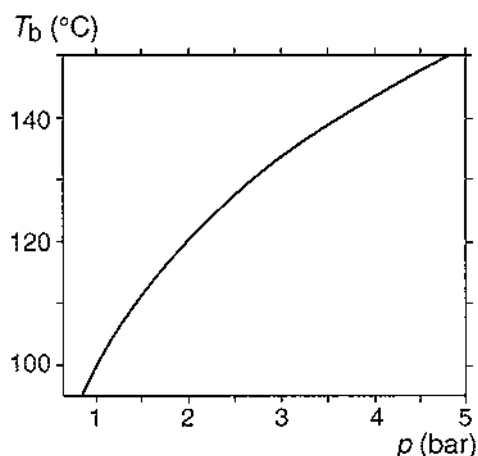[c] Falling film.

FIGURE 6.21 Boiling temperature (T_b) of water as a function of pressure (p).

the higher α will be. This is because a higher Re causes the laminar surface layer to be thinner. Turbulent flow of fluids past a wall may be characterized by

$$\text{Nu} \approx 0.027\ \text{Re}^{0.8}\ \text{Pr}^{0.33} \tag{6.18}$$

where

$$\text{Nu} \equiv \alpha\ d/\lambda \tag{6.18a}$$

$$\text{Re} \equiv v\ d\ \rho/\eta \tag{6.18b}$$

$$\text{Pr} \equiv c_p\eta/\lambda \tag{6.18c}$$

where v is average linear flow velocity, d is distance between two plates or diameter of a pipe, and η is viscosity of the liquid. Coefficients of heat transfer can be calculated by using these equations together with tabulated data. A difficulty involved is that α depends closely on temperature (mainly because η is temperature-dependent). Furthermore, for the variables in Equation (6.18) the values at the wall, where the temperature is not precisely known, should be inserted.

   α can have other values under other conditions, e.g., for condensing steam. Usually, calculating the total heat transfer is not a simple task, especially when no forced convection is generated (rapid forced circulation). Even such circulation as happens in heat exchangers of the common type causes problems in calculation, e.g., because the temperature difference involved may not be constant throughout the processing. In a falling film evaporator, the liquid velocity in the milk film v, and thereby Re, strongly depends on conditions, especially viscosity. Another

difficulty is that the total coefficient of heat transfer may decrease during processing, due to fouling of the equipment, i.e., deposition of a layer of milk components.

SUGGESTED LITERATURE

- Principles of heat transfer are discussed in most texts on food engineering, e.g.:

    R. P. Singh and D. R. Heldman, *Introduction to Food Engineering*, Academic Press, Orlando, 1984, which gives a fairly elementary general discussion.

- Heating processes used for milk are extensively discussed by:

    H. Burton, *Ultra-High-Temperature Processing of Milk and Milk Products*, Elsevier, London, 1988.

- Effects of heat treatment on milk are extensively discussed in:

    P. F. Fox, ed., *Heat-Induced Changes in Milk*, 2nd ed., International Dairy Federation, Brussels, 1995.

7

Centrifugation

Centrifugal separation is applied for the following reasons:

 a. To obtain cream and/or skim milk
 b. In the separation of whey or sweet-cream buttermilk
 c. To standardize milk and milk products to a desired fat content

Another purpose may be to clean the milk, essentially to remove dirt particles, leukocytes, etc. Centrifugal separation can also be applied to remove bacteria as well as their spores (''bactofugation'').

There are various types of milk separators or centrifuges. Figure 7.1 illustrates the basic principle of one of these; skim milk and cream are discharged through centripetal pumps, into which the high-speed liquids are forced. The fully hermetic separator has packing rings between its rotating and stationary parts; hence, the liquid flows through a closed circuit. A separator acts as a pump for the discharged liquids.

The most essential objective of the separation process is its completeness, i.e., a low fat content in the skim milk. The factors affecting the proportion of the fat that is left in the skim milk can be discussed with reference to Equation 7.1, describing the velocity v of a fat globule of diameter d in the centrifugal field:

$$v = \frac{R\,\omega^2(\rho_p - \rho_f)\,d^2}{18\,\eta_p} \tag{7.1}$$

where R = effective radius of the centrifuge, ω = angular velocity of the rotation (in radians per second), ρ = density, and η = viscosity; subscripts p and f refer
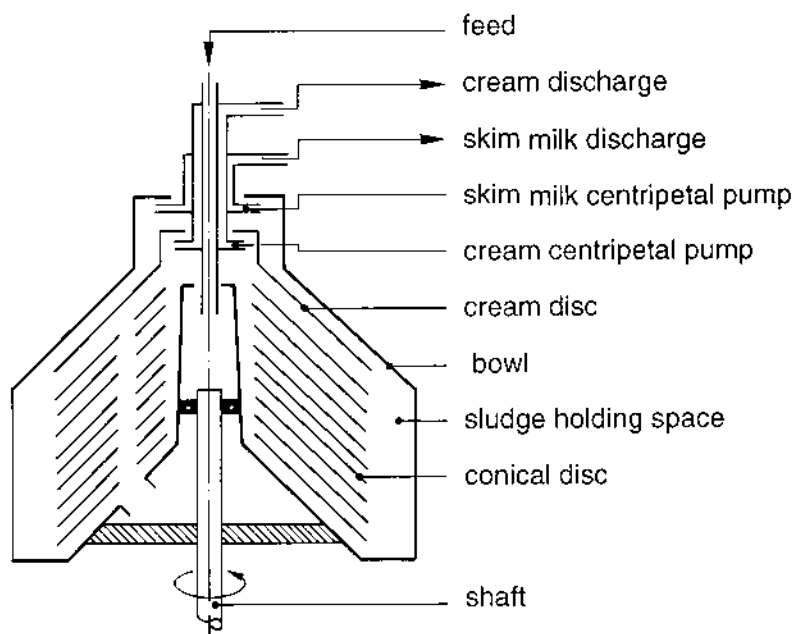
FIGURE 7.1 The basic principle of a so-called half-hermetic milk separator. The (revolving) bowl and the (nonrevolving) machinery for supply and discharge are drawn. In reality, the bowl contains a far greater number of discs.

to plasma and fat globules, respectively. The following are the main factors determining the creaming efficiency:

   a. *The centrifugal acceleration $R\omega^2$*, usually about 4000 g, where g is the acceleration due to gravity

   b. *The distance over which the fat globules must move*. Figure 7.1 illustrates that discs divide the room in the separator into a great number of spacings. The separation therefore occurs over only about 0.5 mm.

   c. *Time available for separation*. This results from the volume of the part of the centrifuge in which separation occurs and from the flow rate.

   d. *The size distribution of the fat globules*. The critical diameter of fat globules that are just recovered by centrifugation is about 0.7 μm. This can be deduced from the constructional details of the separator, the operational conditions, and the properties of milk, by using Equation 7.1. Accordingly, the amount of fat present in small fat globules is
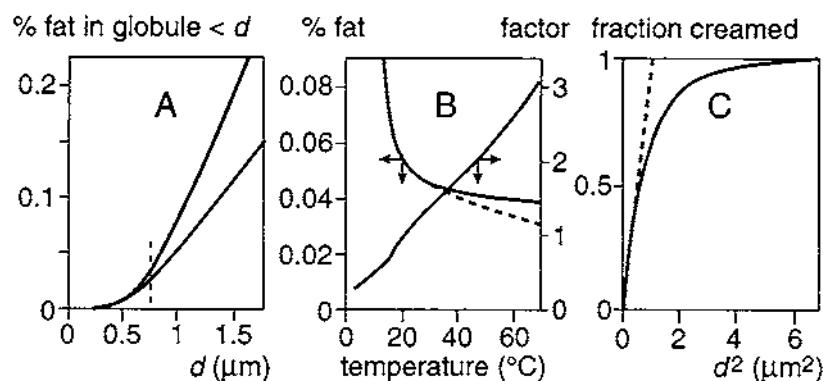
FIGURE 7.2 Centrifugal separation. (A) Amount of fat present in globules smaller in diameter than d; the curves represent approximately the extremes found in milks from individual cows. (B) Influence of the separation temperature on the efficiency factor (see text) and on the fat content of separated milk; the broken line is expected if no disruption of fat globules would occur. (C) The fraction of the fat not left in the skim milk, as a function of the square of the diameter of the fat globules; the broken line is expected if the operational conditions would not vary. Approximate examples. Partly after P. Walstra and H. Oortwijn, *Neth. Milk Dairy J.* **29** (1975) 263.

paramount. This is shown in Figure 7.2A. In addition, some nonglobular fat is present in milk (about 0.025%). All in all, at 45°C a fat content in the separated milk of 0.04% to 0.05% can usually be obtained.

e. *Temperature.* Above all, it affects η_p, but also ρ_f, ρ_p, and, slightly, d. These variables can be lumped in an efficiency factor, i.e., the velocity calculated from Equation 7.1 divided by the velocity at 20°C; this factor, together with the fat content of the separated milk, is shown in Figure 7.2B. If milk is to be separated at a low temperature, say 4°C, a specially constructed separator (cold milk separator) may be used, where the fat content of the resulting skim milk mostly amounts to 0.07% to 0.1%. This poses no problem if the cream is only separated to standardize the milk.

f. *Fat content of the milk.* The higher the fat content, the less accurate Equation 7.1 is, and the higher the fat content of the skim milk resulting.

g. *Proper operation of the separator*, which implies no vibrations, no leakage, etc. The construction considerably affects the result because it determines the range in holdup time and the effective radius; Figure

7.2C illustrates its influence. If the operational conditions do not vary, the fraction of the fat globules that is removed is proportional to the square of their diameter, as follows from Equation (7.1).

Some further objectives of the separation process are as follows:

a. *Adjusting the fat content of the cream.* This is usually achieved by varying the back pressure in the cream discharge tube and along with that the amount of cream; likewise, the back pressure in the skim milk discharge tube can be varied. A higher than 50% fat cream is usually hard to obtain. If high-fat cream is to be made, the cream can be resepa-rated in a specifically designed centrifuge, often called a concentrator.

b. *Preventing damage to fat globules* (see also Section 5.3.2.4). Such damage can especially occur in the cream. Some disruption of fat glob-ules can occur in the separator itself, especially at temperatures above 40°C. Because of this, an increase of the separation temperature above 50–60°C only slightly improves the creaming efficiency (see Fig. 7.2B).

c. *Long-term continuous use.* Operation time may be limited because the sludge holding space becomes filled. Self-desludging centrifuges are generally used, from which the sludge (separated dirt particles, large casein micelles, leukocytes, microorganisms) can be removed without stopping the separator. Sludge is eventually also deposited, however, between the discs, causing the separating efficiency to be impaired. To limit the amount of sludge, the milk can be filtered.

A different disc stack may be fitted if the separator is only used to clean the milk. It allows operation at double capacity and at low temperature.

BACTOFUGATION

Bacteria, and especially bacterial spores, can be separated at very high centrifugal force and high temperature in a specifically designed centrifuge, called a *bacto-fuge*. A double treatment at approximately 73°C leads to three decimal reductions, slightly more for bacterial spores. The method is expensive, partly because a small percentage of the milk solids becomes incorporated in the sludge. The sludge is removed continuously and often is readded to the milk after sterilization. It has been suggested that the method be used for the removal of spores of *Bacil-lus cereus* from pasteurized beverage milk. It is employed to reduce the number of spores of *Clostridium tyrobutyricum* in cheese milk.

8

Homogenization

Homogenization of milk causes disruption of milk fat globules into smaller ones. The milk fat–plasma interface is thereby considerably enlarged, usually by a factor of 5–10. The new interface is rapidly covered with milk protein, predominantly casein.

8.1 OBJECTIVES

Homogenization is applied for any of the following reasons.

a. *Counteracting segregation, for the most part creaming*. To achieve this, the size of the fat globules should be greatly reduced. Another example is the sedimentation of cocoa particles; the homogenizer can reduce even these particles. A cream layer or sediment in the product may be a nuisance for the user, especially if the package is nontransparent.

b. *Improving stability towards partial coalescence*. The increased stability of homogenized fat globules is caused by the reduced diameter and by the acquired surface layer of the fat globules. Moreover, partial coalescence especially occurs in a cream layer, and such a layer forms much more slowly in homogenized products. All in all, prevention of partial coalescence usually is the most important purpose of homogenization; a cream layer per se is not very inconvenient because it can readily be redispersed in the milk.

c. *Creating desirable rheological properties*. Formation of homogenization clusters (Section 8.7) can greatly increase the viscosity of a prod-

uct, e.g., cream. Homogenized and subsequently soured milk (e.g., yogurt) has a higher viscosity than unhomogenized milk. This is because the fat globules that are now partly covered with casein participate in the aggregation of the casein micelles.

d. *Recombining milk products*. A homogenizer, however, is not an emulsifying machine. Therefore, the mixture concerned should first be pre-emulsified, e.g., by vigorous stirring; the formed coarse emulsion is subsequently homogenized. (See Section 17.9.)

8.2 OPERATION OF THE HOMOGENIZER

Homogenizers of the common type consist of a high-pressure pump that forces the liquid through a narrow opening, the so-called homogenizer valve. Figure 8.1A gives a flow chart; for the moment, we will leave the second stage aside. The principle of the operation of the valve is illustrated in Figure 8.1B. The valve has been dimensioned in such a way that the pressure in the valve (p_2) equals 0 at a reasonable homogenizing pressure ($p_{hom} = p_1$), e.g., $p_{hom} > 3$ MPa. Actually, p_2 tends to become negative, which implies that the liquid can start boiling; in other words, cavitation can occur. (Cavitation is the sudden formation and collapse of vapor bubbles caused by pressure fluctuations.)

During homogenization, the liquid upstream of the valve has a high potential energy. On entering the valve, this energy is converted to kinetic energy (according to the rule of Bernoulli). The high liquid velocity in the very narrow opening in the valve leads to very intense turbulence; the kinetic energy of the liquid now is dissipated, i.e., converted to heat (thermal energy). Only a small part of the kinetic energy, <1%, is used for globule disruption, i.e., for conversion to interfacial energy. The net amount of energy dissipated per unit volume (in $J \cdot m^{-3}$) numerically equals p_{hom} (in $N \cdot m^{-2}$); if the specific heat of the liquid is c_p, the temperature rise as caused by homogenization will be p_{hom}/c_p. For milk, $c_p \approx 4 \times 10^6 \, J \cdot m^{-3} \cdot K^{-1}$; thus $\Delta T \approx p_{hom}/4$ if p_{hom} is expressed in MPa and T in K.

The passage time of the liquid through the valve is very short, i.e., <1 ms. As a result, the average power density $\bar{\epsilon}$ (= energy dissipated per unit volume and per unit time, e.g., in $J \cdot m^{-3} \cdot s^{-1} = W \cdot m^{-3}$) is extremely high: 10^{11}–10^{12} $W \cdot m^{-3}$; see Figure 8.1B. Such high power densities lead to very intense turbulence; this implies that the flow pattern shows very small eddies in which high liquid velocity gradients occur. These eddies thereby cause pressure fluctuations, which can disrupt particles, especially droplets (see Section 8.3).

Figure 8.2 schematically depicts the simplest homogenizer valve applied. The pressure is adjusted by letting the control spring press down the valve with an appropriate stress; hydraulic rather than mechanical pressure may be used. These also are mechanical aids for absorbing pressure fluctuations, which result
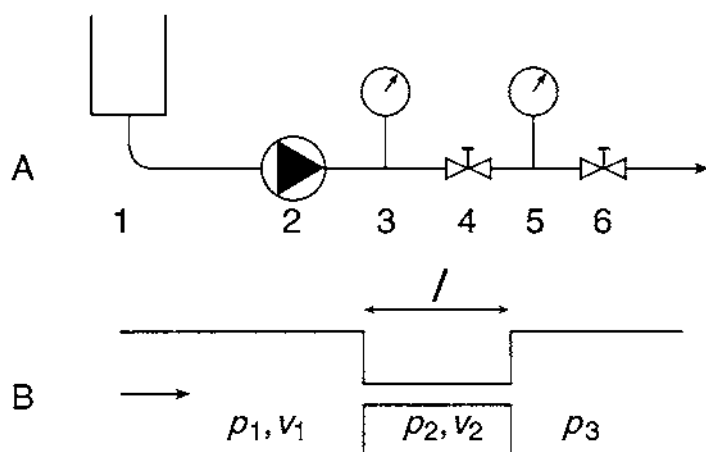
$$p_1 + \tfrac{1}{2}\rho v_1^2 = p_2 + \tfrac{1}{2}\rho v_2^2 \qquad (8.1)$$

Bernoulli:

$$p_1 = p_{hom} \qquad p_2 \approx 0 \qquad p_3 \approx 1\ \text{bar}$$

$$v_1 \ll v_2$$

Hence

$$p_{hom} = \tfrac{1}{2}\rho v_2^2 \qquad (8.2)$$

Power density

$$\varepsilon = p_{hom} / t_p$$

$$t_p \approx l / v_2 \propto l / (p_{hom})^{1/2} \qquad (8.3)$$

Hence

$$\varepsilon \propto (p_{hom})^{3/2} \qquad (8.4)$$

Example:

$$p_{hom} = 200\ \text{bar} = 2 \cdot 10^7\ \text{Pa}$$

hence

$$v_2 = 200\ \text{m·s}^{-1}$$

Suppose

$$l_{eff} = 0 \cdot 01\ \text{m}$$

then

$$t_p = 5 \cdot 10^{-5}\ \text{s}$$

and

$$\varepsilon = 4 \cdot 10^{11}\ \text{W·m}^{-3}$$

Figure 8.1 Operation of the high-pressure homogenizer. (A) Flow diagram: 1, tank; 2, high-pressure pump; 3, 5, manometer; 4, homogenizer valve; 6, valve of second stage; 5 and 6 are not always present. (B) Principle of the homogenizer valve; highly schematic example. p, pressure; ρ, mass density; v, liquid velocity; t_p, passage time; l, passage length.
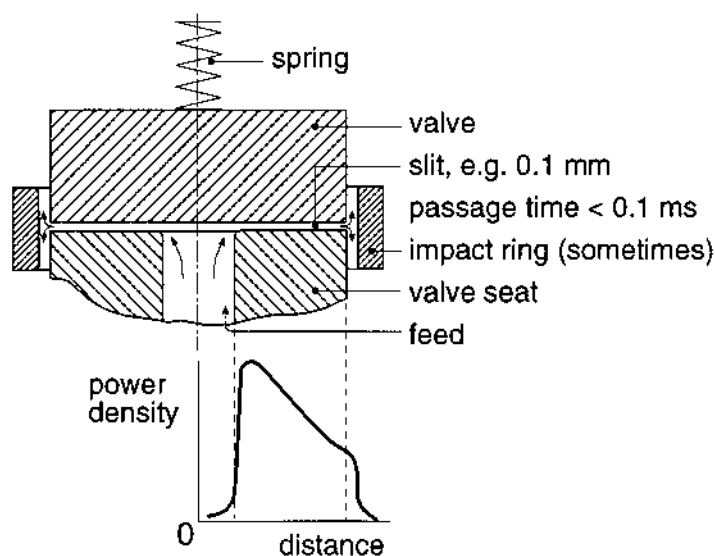
FIGURE 8.2 Cross section of a flat homogenizer valve; near to scale, but the spacing between valve and seat is much smaller than drawn. The supposed course of the power density as the liquid passes through the valve is also shown.

from the high-pressure pump acting with plungers, which produce a somewhat fluctuating flow rate. Note that the initial power density is very high and that it decreases during passage of the liquid through the valve because the liquid velocity is inversely proportional to the distance to the center of the valve. On leaving the valve, the liquid decreases sharply in velocity, hence in power density.

Let the example of Figure 8.2 refer to a homogenizer with a capacity of $8000 \text{ L} \cdot \text{h}^{-1}$. In the tube upstream of the valve, the Reynolds number (Re) is about 80 000 for milk of 40°C. It can be deduced that the maximum Re in the valve has the same value; in other words, Re does not depend on the homogenizing pressure as long as the flow rate is the same. Remember that Re $= h\rho v/\eta$, where h is a characteristic length scale and η is viscosity. The width of the valve slit h is smaller for a higher pressure, but v_2 is inversely proportional to h; see also Equation (8.2). In our example, for a pressure of 20 MPa we calculate a slit width of about 0.2 mm and a passage time of about 0.3 ms.

Sometimes an impact ring is present. At that point, the liquid velocity is still relatively high, say, $50 \text{ m} \cdot \text{s}^{-1}$ ($=180 \text{ km} \cdot \text{h}^{-1}$). Some solid particles can thereby be disrupted, at least if the particle density is higher than the liquid density. This is a much used method for reducing the size of cocoa particles in chocolate milk.

Most homogenizer valves are more complicated than the one represented in Figure 8.2. Usually, they are more or less conically shaped. More sophisticated surface reliefs occur as well. To prevent uneven wear, the valve in some homogenizers is rotated relative to the valve seat.

8.3 THEORY OF KOLMOGOROV

As stated above, intense turbulence occurs in the homogenizer valve. That means that there are eddies. The largest eddies have a diameter similar to the slit width, and they transmit their kinetic energy to smaller eddies, these in turn to still smaller ones, and so on until the friction in the liquid becomes so large as to convert the kinetic energy into heat. If the Re is high, e.g., $>40\,000$, the turbulence on the scale of the smaller eddies is isotropic, i.e., it is the same in all directions. For this situation, Kolmogorov has developed a very useful theory that shows that the turbulence only depends on the power density, ϵ. (Sometimes ϵ is called energy density, but this is an incorrect designation.) Of course, the relative velocity in an eddy is on average 0, but the root-mean-square velocity difference is not; it depends on the distance x over which the difference is considered, according to

$$\langle v^2(x) \rangle \approx \epsilon^{2/3} x^{2/3} \rho^{-2/3} \tag{8.5}$$

According to the rule of Bernoulli (see Fig. 8.1), pressure differences occur, given by

$$\langle \Delta p(x) \rangle \approx \rho \, \langle v^2(x) \rangle \tag{8.6}$$

These pressure differences fluctuate rapidly with time and among sites in the valve. In principle, they can disrupt a droplet; this happens if the pressure difference surpasses the Laplace pressure p_L of the droplet, which is given by

$$p_L = 4\,\gamma/d \tag{8.7}$$

where d = droplet diameter and γ = interfacial tension (in our case, γ mostly is between 15 and 28 mN $\cdot$ m^{-1}). By putting $x = d$, the combination of Equations (8.5), (8.6), and (8.7) yields the diameter d_{max} of the largest droplets that cannot be disrupted by the turbulent eddies involved:

$$d_{max} \approx \epsilon^{-0.4} \gamma^{0.6} \rho^{-0.2} \tag{8.8}$$

We thus have a very simple relationship. The average globule size is usually assumed to be proportional to d_{max}. Equation (8.8) then also holds for that average diameter, albeit with a smaller proportionality factor. Generally, the theory of Kolmogorov gives scaling laws, but the proportionality constants in the equations are of the order of unity.

The theory of Kolmogorov is very successful. Note that the viscosities of

the continuous (η_c) and dispersed (η_D) fractions do not appear in Equation (8.8). This stands to reason for η_c; after all, the disruption of the droplets is due to pressure fluctuations, i.e., inertial forces, rather than to shear stresses (that are proportional to viscosity). On the other hand, the droplet viscosity η_D may affect the result; in other words, Kolmogorov's theory would need some adaptation (see also below). Furthermore, the above only holds under certain conditions. For example, an important prerequisite is that the new droplets not be smaller than the smallest eddies, the size of which is given by the so-called Kolmogorov scale:

$$\lambda_0 \approx \eta_c^{0.75} \, \rho^{-0.5} \epsilon^{-0.25} \tag{8.9}$$

As stated above, on a still smaller scale the kinetic energy is converted to frictional heat.

The above is a partial description of what happens in the valve. When a droplet is disrupted into smaller ones, the oil–water interface increases in area. It has to be covered by surface-active substances (i.e., proteins in milk) because otherwise two globules that encounter each other may coalesce again. All this is illustrated in Figure 8.3, rows 1–4. The transport of material to the droplet is not caused by diffusion but by convection; it is enormously enhanced by the intense turbulence. Kolmogorov's theory shows that the flux J of particles (e.g., protein) to a droplet of radius r is given by

$$J \approx 2.6 \, N(r + r_p)^3 \epsilon^{1/2} \eta_c^{-1/2} \tag{8.10}$$

where r_p = radius of the protein molecule (or protein particle) and N = number concentration of particles. From Equation (8.10) the approximate adsorption time t_a (i.e., time needed for coverage of the droplet with protein) can be calculated. The turbulence causes the droplets to frequently encounter each other and such an encounter may well imply collision. The average time between two encounters (t_e) is given by

$$t_e \approx d^{2/3} \rho^{1/3}/15 \, \phi \epsilon^{1/3} \tag{8.11}$$

where ϕ = volume fraction of the emulsion droplets. If $t_a > t_e$, some recoalescence will occur in the valve; consequently, the average globule size obtained is larger than the diameter calculated by Equation (8.8). Another important variable involved is the time needed for deformation, hence disruption of the droplet, t_d. It is given by

$$t_d \approx \eta_D/5 \, \epsilon^{2/3} d^{2/3} \rho^{1/3} \tag{8.12}$$

If t_d is going to be relatively large, disruption will be more difficult and the resulting drops larger than predicted by Equation (8.8). Obviously, the effect is stronger if η_D is higher.

The following are some approximate examples of these characteristic times (homogenization pressure ≈ 20 MPa):
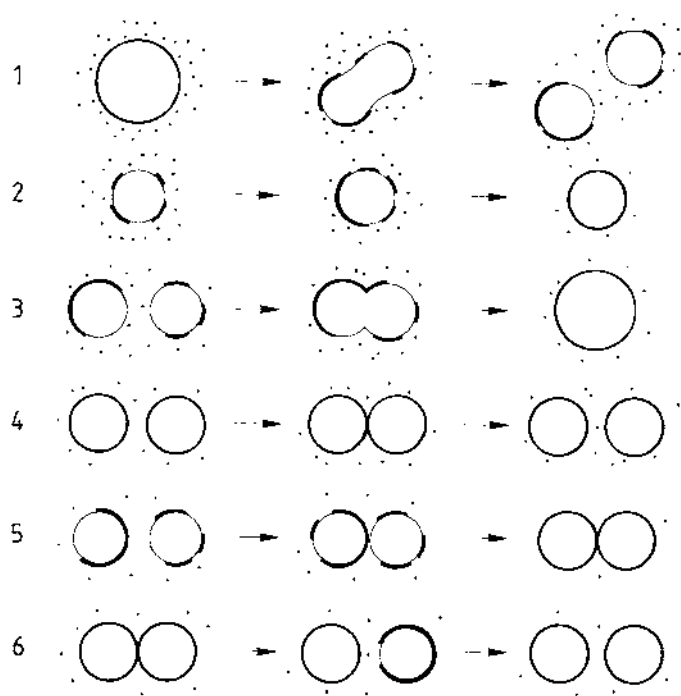
FIGURE 8.3 Processes occurring in a water–oil emulsion during homogenization. The droplets are depicted by thin lines, the surface-active material (e.g., protein) by thick lines and dots. Highly schematic and not to scale.

	Milk 4% fat	Cream 25% fat
Adsorption time (t_a, μs)	0.25	1
Encounter time (t_e, μs)	0.15	0.02
Deformation time (t_d, μs)	0.3	0.3

For milk, all of these times are approximately the same, but for cream they are certainly not. Furthermore, all of the times mentioned are very short, i.e., by a factor of about 100 shorter than the passage time through the homogenizer valve.

Table 8.1 and Figure 8.4 give examples of the effects created by homogenization. Since the conditions, hence ϵ, vary from place to place in the homogenizer valve, there will be considerable spread in d_{max}. Accordingly, the resulting size distribution is fairly wide as is shown in, for instance, Figure 8.4. Repeated homogenization reduces the average diameter d_{vs} as well as the distribution width

TABLE 8.1   Average Effect of Homogenization on Milk With 4% Fat[a]

Homogenization pressure (MPa)	–	5	10	20	40
Temperature rise (K)	—	1.2	2.5	5	10
Diameter of smallest eddies (μm)	—	0.32	0.24	0.18	0.14
Number of fat globules (μm^{-3})	0.015	2.8	6.9	16	40
d_{vs} (μm)	3.3	0.72	0.47	0.31	0.21
d_{max} (μm)[b]	9	3.1	2.3	1.6	1.1
A (m^2 · ml^{-1} milk)	0.08	0.37	0.56	0.85	1.3
c_s	0.44	0.89	0.85	0.83	0.82
H (μm^2)	20	2.2	0.87	0.36	0.16

[a] Approximate results. See Section 3.1.1.1 for definition of variables.
[b] 99% of the fat is in globules $<d_{max}$.
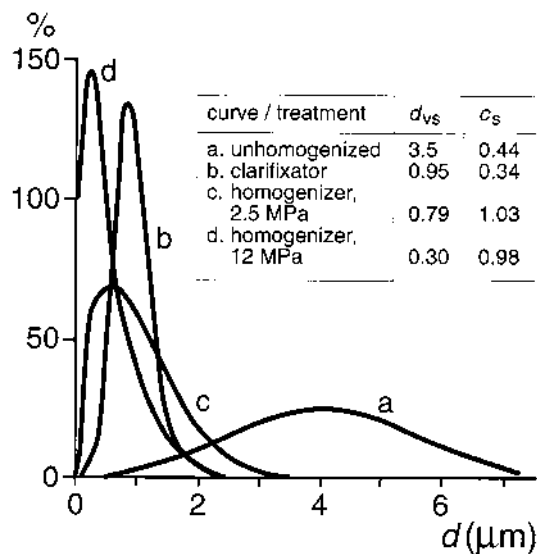
FIGURE 8.4   Some examples of the size distribution of the fat globules in homogenized and unhomogenized milk. The volume frequency (% of the fat per micrometer class width) as a function of globule diameter. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

c_s. (Particle size distributions are described in Section 3.1.1.1.) The distribution also becomes wider if significant coalescence of droplets occurs.

8.4 FACTORS AFFECTING THE FAT GLOBULE SIZE

a. *Type of homogenizer*, especially construction of the homogenizer valve. For the same p, the passage time t_p will differ and and so does $\bar{\epsilon} = p/\bar{t}_p$. Moreover, the spread in conditions will differ. All in all, considerable variation in results is observed. Figure 8.5 gives examples.

b. *Homogenizing pressure*. Equation (8.4) shows that $\bar{\epsilon} \propto p^{1.5}$. From $d_{max} \propto \epsilon^{-0.4}$ [Eq. (8.8)] and from the observation that the shape of the size distribution of the fat globules depends only a little on the pressure, it can be inferred that

$$\log d_{vs} = \text{constant} - 0.6 \log p \qquad (8.13)$$

and that

$$\log H = \text{constant} - 1.2 \log p \qquad (8.14)$$

where sedimentation parameter H is an average diameter squared (see also Figure 8.5). These are useful equations because from the result obtained at one pressure the effect of other pressures can be deduced.
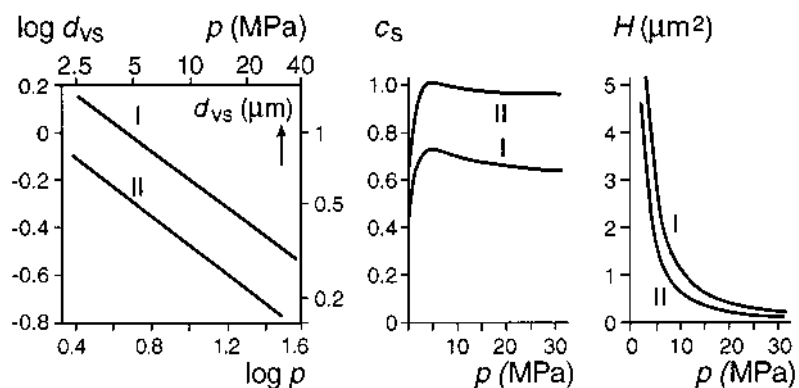
FIGURE 8.5 Effect of homogenization pressure (p) on average fat globule size (d_{vs}), relative distribution width (c_s), and sedimentation parameter (H) for two homogenizers (I and II). (From H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

The constant depends on the homogenizer and some other parameters. Table 8.1 gives examples of results.

c. *Two-stage homogenization*. The milk first passes the ''usual'' homogenizer valve by which the pressure is reduced from, for instance, 20 to 5 MPa (the minimum pressure inside the valve equals zero). By the second homogenizer valve the pressure is reduced to about 1 bar (0.1 MPa); see Figure 8.1. There is no significant homogenization in the second valve slit. Accordingly, the second-stage influence on the fat globule size is small. In other words, one-stage homogenization at 20 MPa leads to a result similar to that in two stages at 20 and 5 MPa, respectively. The result is worse if the pressure drop in the second stage increases to more than about 30% of the total pressure drop. The purpose of two-stage homogenization is another one (see Section 8.7).

d. *Fat content and ratio of amount of surfactant (usually protein) to amount of fat*. If insufficient protein is available to cover the newly formed fat surface, the average diameter of the fat globules (d_{vs}) and the relative distribution width (c_s) will be larger (see Fig. 8.6). This results, at least qualitatively, from the theory given in Section 8.3. In cream, the time needed for formation of adsorption layers is longer than in milk; on the other hand, the average time between encounters
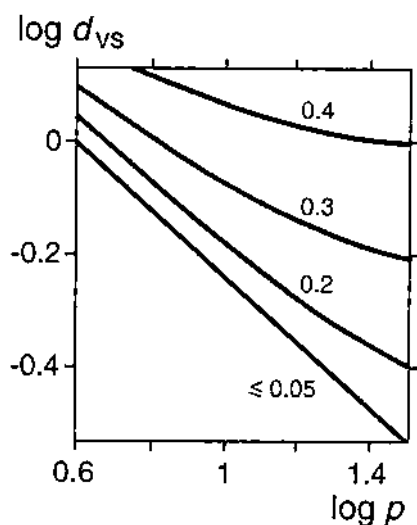

FIGURE 8.6 Effect of homogenization pressure (p, in MPa) on average fat globule diameter d_{vs} (μm) of milk and cream. The fat content, expressed as volume fraction, is near the curves. After P. Walstra and G. Hof, unpublished.

of one droplet with another is much shorter. As a result, in cream far more recoalescence of newly formed droplets can occur. Figure 8.6 also shows that the simple relationship of Eq. (8.13) is lost if the fat content surpasses, say, 10% (see also Section 8.7).

e. *Temperature*. Homogenization is usually done at temperatures between 40°C and 75°C. Homogenization is poor if the temperature is so low that part of the fat is crystalline (see Figure 8.11). Further increase of the temperature still has a small effect, presumably because the viscosity of the dispersed fraction decreases somewhat.

f. *Proper operation of the homogenizer*. A varying pressure (caused by leaking valves, etc.), a worn homogenizer valve, and air inclusion all may work out badly. Air inclusion and wear of the homogenizer valve should be rigorously avoided. If the liquid contains solid particles like dust or cocoa, the valve may quickly wear out, resulting in unsatisfactory homogenization.

The effect of the homogenization should be checked frequently. The average fat globule size may be derived from specific turbidity measurements at long wavelength (e.g., 1 μ) after the milk has been diluted and the casein micelles dissolved. In this way, the homogenizing effect can be evaluated rapidly and simply. In principle, continuous determination is possible. In actual practice, however, an accelerated creaming test is usually done. A certain quantity of milk is centrifuged and the fat content of the resulting skim milk determined.

8.5 SURFACE LAYERS

Figure 8.7 illustrates the surface layer of milk fat globules formed by homogenization. The newly formed membranes are predominantly composed of micellar casein and serum protein. Some of the casein micelles in the layer are present as such, but most are more or less spread out into micelle fragments or a layer of submicelles. The spreading occurs if a micelle touches a denuded oil–water interface. (It is sometimes assumed that the micelles are disrupted by the homogenizer and that the resulting micelle fragments are subsequently adsorbed onto the fat globules, but this hardly occurs; compare the size of the micelles with the scale of the smallest eddies in Table 8.1.) The spreading occurs in a time scale of the order of 0.1 μs; adsorption times are mentioned in Section 8.3.

Equation (8.10) gives the flux of protein particles to the fat droplet during homogenization. Assuming that the protein concentration c_p is proportional to $N_p r_p^3$, it can be deduced that the increase of the surface excess of protein (Γ, in mass of protein per unit surface area) with time is given by

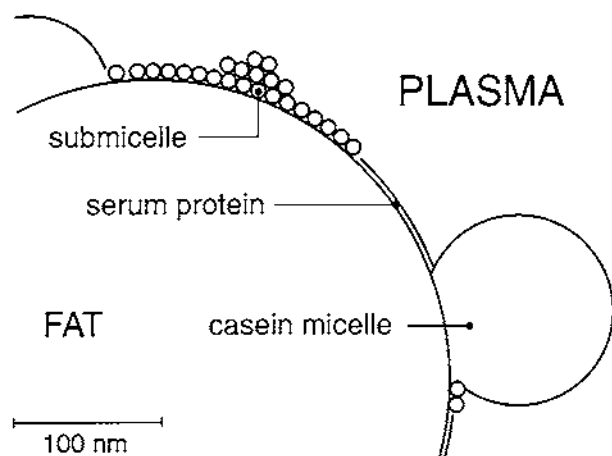$$d\Gamma/dt \propto c_p r(1 + r_p/r)^3 \tag{8.15}$$

FIGURE 8.7 New surface layer of fat globules formed during homogenization. Highly schematic. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

The factor between brackets is highly variable. For casein micelles, r_p ranges from 60 to 150 nm; for serum proteins, $r_p \approx 2$ nm; for the fat globules, r ranges from some 100 to 400 nm. That means that the factor may vary between 1.02 and 4, and that it is large for small droplets encountering casein micelles; it virtually equals unity for fat globules with serum proteins. This has some important consequences:

    a.   Casein micelles are preferentially adsorbed. Casein makes up about 80% of the protein in milk plasma, but about 93% of the protein in the new surface layers; because the ''casein layer'' is thicker than the serum protein layer, casein covers some 75% of the surface area of the fat globules. These results apply for homogenizing at about 10 MPa.

    b.   Large micelles are adsorbed preferentially over small ones.

    c.   The differences in adsorption among protein (particles) are largest for smaller fat globules, since then $r \approx r_p$ for some micelles. That explains why smaller fat globules usually have a thicker protein layer than large globules (see Fig. 8.8C). When homogenization pressure is very high (say, >30 MPa), the surface layers obtained are virtually devoid of serum protein.

A rough average for Γ in homogenized milk is 10 mg $\cdot$ m^{-2}. The following are also factors that affect Γ:

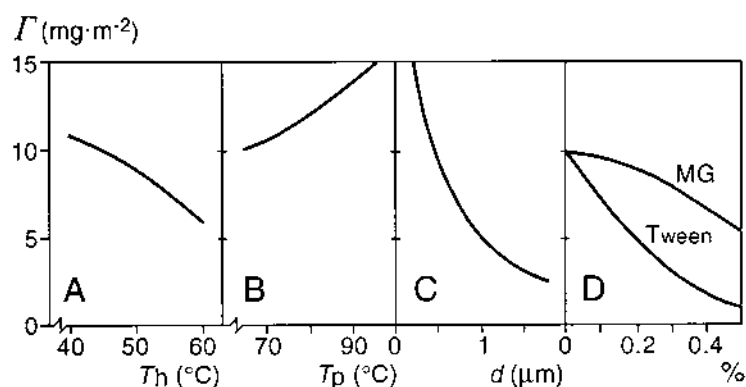   a.   *Homogenization temperature*. This is explained by the casein micelles

FIGURE 8.8 Effect of some process variables on the protein surface excess Γ of the fat globules in milk (or cream). Homogenization pressure about 10 MPa. Approximate examples. (A) Homogenization temperature (T_p was 70°C). (B) Pasteurization temperature, heating during 10 min (T_h was 40°C). (C) Fat globule diameter; it concerns globules in one sample of homogenized milk. (D) Addition of a water-soluble (Tween) or an oil-soluble (monoglycerides, MG) surfactant to 12% fat cream before homogenizing.

    spreading more rapidly over the fat–water interface at, say, 70°C than at 40°C, which causes Γ to decrease (Fig. 8.8A).
b. *Preheating*, e.g., 20 min at 80°C. This causes serum proteins to associate with the casein micelles. Consequently, Γ increases (see Fig. 8.8B) because formation of a thin local layer of serum proteins is no longer possible.
c. In evaporated milk the casein micelles may have coalesced into larger ones. This also causes Γ to increase.
d. Instead of protein, surfactants like monoglycerides or Tweens may adsorb and thereby lower Γ (see Fig. 8.8D). After all, small molar mass surfactants tend to reduce the surface tension much further than most of the proteins do; hence, they are preferentially adsorbed. They can also displace proteins adsorbed at the oil–water interface when added after homogenization.

8.6 STABILITY

Plasma protein, predominantly casein, covers a large part (up to about 90%, in recombined milks 100%) of the surface area of homogenized fat globules. This makes the globules behave to some extent like large casein micelles. Any reaction

that causes casein micelles to aggregate, such as renneting, souring, or heating at very high temperatures, will also cause the homogenized fat globules to aggregate. Moreover, aggregation will take place more quickly because homogenization has increased the apparent casein concentration (i.e., the casein concentration effective in aggregating). This is discussed in Section 3.2.3.6. The effect is stronger when fat content and homogenizing pressure are higher (see, e.g., Fig. 8.9).

The following are some important consequences of the altered surface layer:

a. Heat stability is decreased, as illustrated in Figure 8.9. This is further discussed in Sections 15.1.2 and 16.1.4. A related aspect is the influence on feathering of cream in coffee (Section 15.1). Addition of small molar mass surfactants (often called emulsifiers) before homogenization may increase stability to heat coagulation (see also Fig. 8.8D).

b. Homogenized milk fat globules generally show a high stability toward partial coalescence because they are so small. Moreover, surface layers that consist fully of plasma protein (as in recombined milk) yield a very high stability to the globules. The addition of surfactants (that displace protein at the oil–water interface; see Figure 8.8D) can considerably decrease the stability to coalescence. This plays a part in a product such as ice cream (Section 15.3).
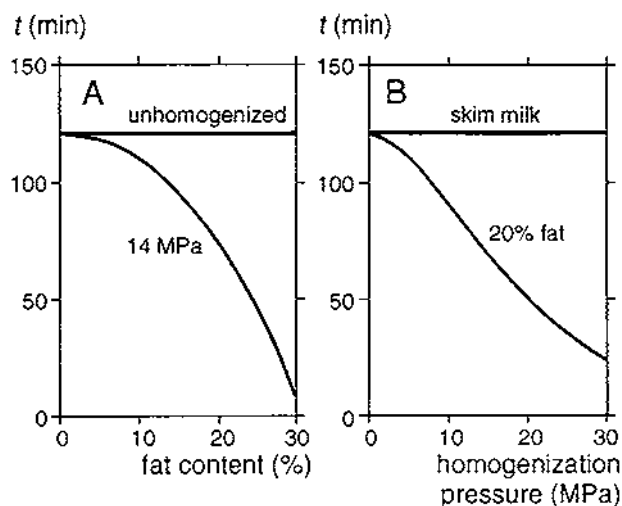

FIGURE 8.9   Time (*t*) needed for coagulation of milk or cream at 120°C as affected by homogenization. After B.H. Webb and G.E. Holm, *J. Dairy Sci.* **11** (1928) 243.

   c.   Sour products (yogurt, sour cream) and cheese made of homogenized milk (or cream) have different rheological properties from those of unhomogenized milk; this is caused by the fat globules becoming part of the casein network. An example is given in Figure 20.5.

8.7 HOMOGENIZATION CLUSTERS

The homogenization of cream usually causes its viscosity to be very much increased, as is shown in Table 8.2. On microscopic examination one sees large agglomerates of fat globules rather than single globules in the homogenized cream. These so-called homogenization clusters contain very many fat globules, up to about 10^5. Because the clusters contain interstitial liquid, the effective volume fraction of particles in the cream is increased, and hence also its (apparent) viscosity. Adding casein micelle–dissolving agents can disperse the clusters. In other words, the fat globules in the cluster are interconnected by casein micelles.

     The formation of homogenization clusters can be explained as follows. During homogenization, when a partly denuded fat globule collides with another globule that has been covered with casein micelles, such a casein micelle can also reach the surface area of the former globule. As a result, both fat globules are connected by a ''bridge'' and form a homogenization cluster (see Figs. 8.3, row 5, and 3.4). The cluster will immediately be broken up again by turbulent eddies (Fig. 8.3, row 6). If, however, too little protein is available to fully cover the newly formed fat surface, clusters are formed from the partly denuded fat globules just outside the valve slit of the homogenizer, where the power density is too low (Fig. 8.2) to disrupt the clusters again.

     In other words, clustering may occur if c_p (=protein concentration, in $kg \cdot m^{-3}$) is less than $\Gamma \Delta A$ (Γ = surface excess, in $kg \cdot m^{-2}$; ΔA = increase in

TABLE 8.2   Effect of One- and Two-Stage Homogenization on Formation of Homogenization Clusters in Cream With 20% Fat

Homogenization[a]	Pressure (MPa)	η[b]	Clusters
Not	0	1	−
1 stage	7	8.9	++
1 stage	21	30.1	++++
2 stages	21/7	4.5	+

[a] Homogenization temperature = 65°C.
[b] Apparent viscosity of homogenized relative to unhomogenized cream.
Source: F. J. Doan, *J. Dairy Sci.* **12** (1929) 211.

surface area, in $m^2 \cdot m^{-3}$); $\Delta A \approx 6 \phi/d_{vs}$ (ϕ = volume fraction of fat). Clearly, the following conditions promote formation of homogenization clusters:

    a. High fat content.
    b. Low protein content.
    c. High homogenizing pressure.
    d. A relatively high surface excess of protein; this is promoted by a low homogenization temperature (less rapid spreading of casein micelles), intense preheating (little serum protein available for adsorption) and, again, a high homogenizing pressure (see Fig. 8.8).

Under practical conditions, clustering due to homogenization does not occur in a cream with less than 9% fat, whereas it always does in a cream with more than 18% fat. At intermediate fat contents, clustering closely depends on pressure and temperature of homogenization (see also Section 15.1.4).

    Clusters can be disrupted again to a large extent (but not fully) in a two-stage homogenizer (Table 8.2). In the second stage the turbulent intensity is too low to disrupt fat globules, and hence to form new clusters, whereas clusters are disrupted; this goes along with some coalescence. Two-stage homogenization of high-fat cream (e.g., 30% fat) insufficiently breaks up homogenization clusters.

8.8   OTHER EFFECTS OF HOMOGENIZATION

Homogenizing milk that contains lipase strongly enhances lipolysis (Section 3.1.5). Raw milk turns rancid within a few minutes after homogenization (see Fig. 8.10A). This should be explained by lipoprotein lipase being capable of
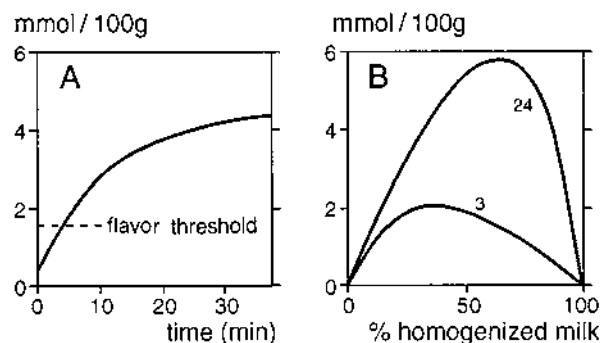

FIGURE 8.10   Influence of homogenization on lipolysis, expressed as acidity of the fat (in mmol per 100 g fat). (A) Raw milk homogenized at 37°C; fat acidity as a function of time after homogenization. (B) Mixtures of raw milk and homogenized pasteurized milk; fat acidity after 3 and 24 h at 15°C. After A. Jellema, unpublished.

penetrating the membrane formed by homogenization, but not the natural membrane. Accordingly, raw milk homogenization should be avoided, or the milk should be pasteurized immediately after homogenization in such a way that the lipase is inactivated. Homogenization is often done before pasteurization, since in the homogenizer the milk may readily be contaminated by bacteria. Furthermore, mixing of homogenized milk with raw milk should be prevented, again to avoid lipolysis (see Fig. 8.10B).

Homogenization of milk has several other effects:

a. The color becomes whiter (Section 3.3.1).
b. The tendency to foam increases somewhat.
c. The proneness to fat autoxidation, and hence to the formation of ensuing off-flavors, is reduced (Section 2.3.3).
d. The fat globules lose their ability to be agglutinated upon cooling (Section 3.1.4.2). This is caused by inactivation of the cold agglutinin rather than by changing the fat globules; homogenization at very low pressure (1 MPa) suffices. Agglutinins (IgM) for bacteria (e.g., *Lactococcus lactis* spp.) can be inactivated also; to achieve this, a higher pressure is required, e.g., 10 MPa.

8.9 CREAMING

An important purpose of homogenization usually is to slow down creaming and thereby to prevent partial coalescence. The purpose is primarily achieved by reducing the fat globules in size. Accordingly, the factors affecting the reduction greatly affect the creaming rate (see Figs. 8.5 and 8.11). The influence of the fat globule size on creaming rate is given by parameter H:

$$H = \sum n_i d_i^5 / \sum n_i d_i^3 \tag{8.16}$$

where n and d are the number and size of the globules, respectively. The largest globules especially contribute to H. That explains why the width of the size distribution can considerably affect H. Different homogenizers lead to different results (Fig. 8.5).

The Stokes velocity [Equation (3.1)] can be used for calculating the rate of cream rising in a product. Table 8.3 gives some examples. The relationship is:

$$q = 4.7(\rho_p - \rho_f)H/\eta_p h \tag{8.17}$$

where q = percentage of fat that reaches the cream layer per day, h = height of the container in cm, and H is expressed in μm^2. But the creaming rate often is slower since (a) the velocity of the globules is decreased due to the presence of other globules, the more so if the fat content is higher; (b) the protein layer

TABLE 8.3 Initial Creaming Rate in Some Milk Products[a]

Product	$(\rho_p - \rho_f)/\eta_p$ $(ks \cdot m^{-2})$	H (μm^2)	h (cm)	q "Stokes" (% per day)	q Corrected[b] (% per day)
Pasteurized milk[c]	50	10–50	20	12–60	8–40
Homogenized milk	50	0.8–1.5	20	0.8–1.7	0.5–1.1
UHT milk	40	~0.4	20	~0.4	~0.2
Evaporated milk	10	~0.4	5	~0.4	~0.1
Sweetened condensed milk	0.2–0.07	4[d]–50	5	0.1–0.3	0.04–0.12

[a] Approximate values of the percentage of the fat reaching the cream layer per day (q).
Creaming of unclustered globules at 20°C in a bottle or tin of height h.
[b] For the influence of fat content and protein layers.
[c] Unhomogenized.
[d] Slightly homogenized.

covering the fat globules after homogenization increases the globule density; and (c) H decreases gradually during creaming because the largest globules reach the cream layer first. Table 8.3 also gives the estimated creaming rate if the latter factors are taken into account. Naturally, the extent of creaming also greatly depends on temperature (see Fig. 7.2) and on possible disturbances by stirring or convection currents.

It goes without saying that the creaming will be much faster when the fat globules are somehow aggregated. The following are causes of aggregation:

a. *Cold agglutination*. Fat globules in most homogenized products cannot flocculate in the cold; however, see Section 8.10, Partial homogenization.
b. *Homogenization clusters*. These are rarely formed, except during homogenizing of cream.
c. *Heating at high temperature* (sterilization) can cause small clusters of homogenized fat globules to be formed, especially in evaporated milk; this is the beginning of heat coagulation.

Figure 8.11 summarizes the effects of process and product variables on properties of homogenized products.

8.10 OTHER WAYS OF WORKING

Partial homogenization is sometimes applied to save on energy and machinery. (A large homogenizer is a very expensive machine and consumes much energy.) The milk is separated into skim milk and cream, and the cream is homogenized and mixed with the separated milk. Two aspects should be considered here:
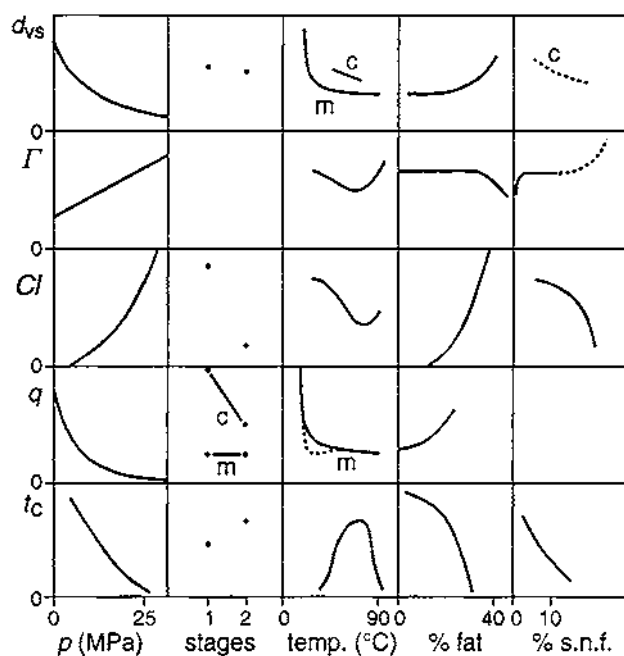
FIGURE 8.11 Effects of conditions during homogenization (p = homogenization pressure) on product properties (m: only for milk, c: cream). d_{vs} = volume/surface average diameter of the fat globules; Γ = protein surface excess; Cl = homogenization clusters in cream; q = creaming rate; t_c = time needed for heat coagulation of cream. Highly schematic. Broken lines are less certain. Homogenization at, for instance, 70°C after preheating at high temperature often leads to a result similar to that of direct homogenization at 90°C. After P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

1. The agglutinin in the skim milk is not inactivated by the homogenization. Accordingly, when low pasteurization is applied, cold agglutination may occur to some extent, which enhances creaming.
2. If the fat content of the cream is too high (e.g., >10%), homogenization clusters are formed, resulting in rapid creaming. But Γ can be kept fairly low by taking the homogenization temperature quite high, say, 70°C (see Fig. 8.8A). As a result, even in a cream with 14% or 15% fat any clustering is prevented (two-stage homogenization).

Several other types of machinery have been devised for homogenizing milk, be it with little success. Sometimes, the ''clarifixator'' is applied. In this milk separa-

tor, the cream collides with obstacles protruding from the cream centripetal pump and is thereby subjected to intense turbulence, which causes a reduction of the fat globule size. Subsequently, the cream is returned to the milk stream. After the fat globules have been sufficiently reduced in size, they escape separation and are discharged in the separated-milk line, which thus actually delivers homogenized milk rather than skim milk. The resulting size distribution of the fat globules is very narrow, but the average size is not very small (see Fig. 8.4).

SUGGESTED LITERATURE

- Homogenization is comprehensively treated in Chapters 9 and 10 of:
    H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.
- Fundamental aspects of emulsions are discussed by:
    P. Walstra, Chapter 2, Formation of emulsions, in P. Becher, ed., *Encyclopedia of Emulsion Technology*, Vol. 1, Marcel Dekker, New York, 1983.
    P. Walstra, Principles of emulsion formation, *Chem. Eng. Sci. 48*, 1993, 333–349.
- Effects of milk homogenization are described by:
    P. Walstra, *Neth. Milk Dairy J. 29*, 1975, 279–294.

9

Concentration Process

9.1 GENERAL ASPECTS

Milk, skim milk, whey, and other milk products can be concentrated, i.e., part of the water can be removed. This is applied to diminish the volume and to enhance the keeping quality. Water can be removed from milk by evaporation. In addition to water, volatile substances, especially dissolved gases, are removed as well. Evaporation is usually done under reduced pressure—hence, decreased temperature—to prevent damage caused by heating. Water can also be removed by reverse osmosis, i.e., high pressure is applied to pass milk through a suitable membrane. Water as well as part (some 1% to 20%, depending on conditions) of some low molar mass substances pass the membrane. A different way of concentrating is by freezing. The more ice crystals are formed, the higher the dry matter content in the remaining liquid. Removal of water to such a low level that the product becomes solid-like is called drying. Drying is achieved by evaporation of water, usually from concentrated milk.

A particular fraction of the dry matter can, of course, be concentrated as well. Examples are cream and syneresed curd. In ultrafiltration, besides water most dissolved components are removed, whereas fat globules, casein micelles, serum proteins, leukocytes, bacteria, and so forth are concentrated. Solutes of a higher molar mass (e.g., citrate) are also concentrated to some extent. We will primarily consider removal of water.

9.1.1 Concentration of Solutes

The degree of concentrating can be defined as the concentration factor Q, i.e., the ratio of dry matter content of the concentrated product to that in the original

material. Consequently, the mass of the concentrated product is $1/Q$ times the mass before concentrating. The concentration of solutes in water increases more than proportionally with Q. Taking the concentration of a substance relative to the amount of water (e.g., in g per 100 g of water), its increase is given by

$$Q^* = Q(1 - D_1)/(1 - QD_1) = D(1 - D_1)/D_1(1 - D) \qquad (9.1)$$

where D_1 is the original dry matter content, expressed as mass fraction. Figure 9.1 illustrates the approximate relationships between Q, Q^*, and D for milk, skim milk, and whey. Q^* becomes very large when D approaches 100%.

During concentrating, some substances can become supersaturated and may precipitate after crystallization. Milk is already saturated with respect to calcium phosphate. As a result of concentrating, the amount of phosphate associated with the casein micelles increases. Lactose becomes saturated in milk at room temperature when $Q \approx 2.8$, but it easily becomes supersaturated. Presumably, lactose will not crystallize at all if milk is concentrated rapidly (as in a spray drier) to a low water content.

But also for substances that remain fully in solution, the thermodynamic activity will not always be proportional to Q^*. For most nonionic species, the activity coefficient increases with decreasing water activity, which implies that
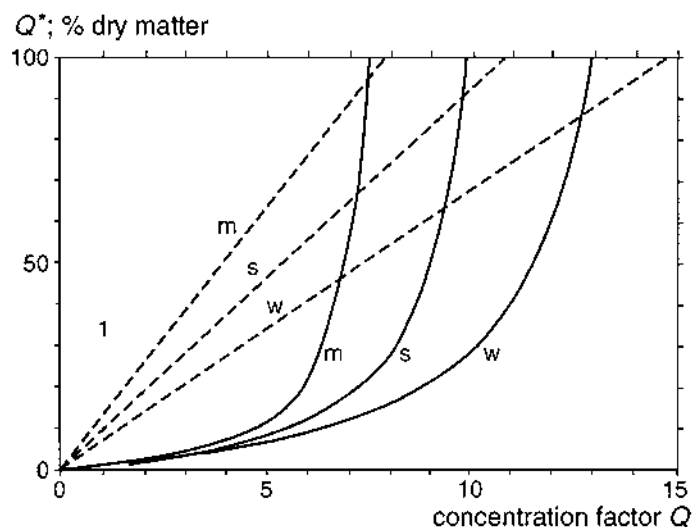
FIGURE 9.1 Percentage of dry matter (---) and relative increase in concentration of substances relative to water (Q^*) (—), as a function of the concentration factor Q for milk (m), skim milk (s), and whey (w) of average composition.

their solubility decreases. For ions, the increasing ionic strength causes a decrease of the activity coefficient. Because of this, the ionization and solubility of salts increase (see Section 2.2.2).

One of the causes for the activity of a solute to become higher than corresponds to $Q*$ is that part of the water is not available as a solvent, which may be designated *nonsolvent water*. A small part of the water is so strongly bound (as water of crystallization or water in the interior of a globular protein molecule) that it cannot be available as a solvent. But negative adsorption of solutes at a surface present (e.g., that of proteins) may be more important. The specific surface area of milk proteins is large, e.g., 10^3 m$^2 \cdot$ g^{-1} for casein (submicelles) in milk, so that the effect may be considerable. Several solutes, especially sugars, exhibit negative adsorption. Figure 9.2 shows that the phenomenon may be interpreted as being caused by steric exclusion, i.e., solute molecules larger than water molecules must stay further away from the surface of protein particles (envisioning the location of solutes to be in their center of gravity), thus leaving a layer of water that is devoid of solute. This explains why the amount of nonsolvent water generally increases with the molecular size of the solute, as illustrated in Figure 9.2.

Knowledge of the amount of nonsolvent water for a particular solute allows conversion of the concentrations in milk into concentrations in plasma, serum,
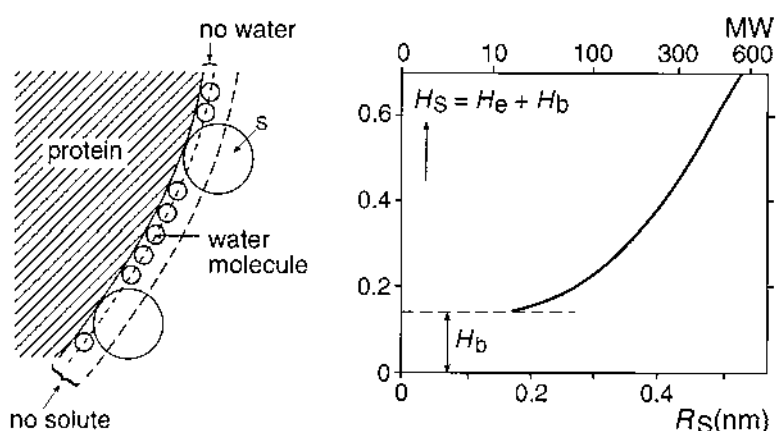
FIGURE 9.2 Steric exclusion of solute molecules (S) at a surface, e.g., of a protein particle. Left: diagrammatic explanation. Right: water not available as a solvent for S (H_s, in g per g of protein), consisting of "excluded" (H_e) and "bound" water (H_b) as a function of radius R_s of the solute molecule. The scale of the molar mass of the solute is approximate. After P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

TABLE 9.1   Conversion of Concentrations of Solutes

If ρ_m =   density of milk, g/ml
   ρ_p =   density of plasma, g/ml
   ρ_s =   density of serum, g/ml
   ρ_w =   density of water, g/ml
   f =   fat content as mass fraction, g/g
   d =   dry matter content as mass fraction, g/g
   p =   protein content as mass fraction, g/g
   c =   casein content as mass fraction, g/g
   x =   content of solute in milk or concentrated milk, in units (g, mol, ml) per kg
The content then is

in milk	x	kg^{-1}	$x\,\rho_m$	L^{-1}
in plasma	$x/(1 - 1.01f)$	kg^{-1}	$x\,\rho_p/(1 - 1.01f)$	L^{-1}
in serum	$x/(1 - 1.01f - 1.08c - hc)$	kg^{-1}	$x\,\rho_s/(1 - 1.01f - 1.08c - hc)$	L^{-1}
relative to water	$x/(1 - d - hp)$	kg^{-1}	$x\,\rho_w/(1 - d - hp)$	L^{-1}

where
  1.01   =   factor to convert fat content to content of fat globules
  1.08   =   factor to convert casein content to content of dry casein micelles (factor
            actually ranging from 1.06 to 1.10)
    h =   factor (in g water/g protein) referring to the amount of nonsolvent water; h
            varies with molar mass of solute. For small molecules (e.g., CO_2) $h \approx 0.15$;
            for lactose $h \approx 0.55$; for serum proteins, average $h \approx 2.6$.

and other products. A formula is given in Table 9.1. Usually, the effect is fairly small for small molecules, but it may be considerable for larger ones. For example, a content of 46 g lactose per kg of milk corresponds to 54.1 g lactose per kg of available water. An amount of 6 g serum proteins per kg milk yields 7.0 g proteins per kg serum, but taking $h = 0$ would lead to 6.45 g serum proteins per kg serum; hence an error of about 8%.

    The above holds only if there is no true (positive) adsorption onto fat globules or casein micelles. Some proteins (lipase, plasmin) do adsorb onto the latter. Some immunoglobulins can adsorb onto fat globules. For various serum proteins, h ranges from 0 to 3.4 (the latter figure refers to β-lactoglobulin).

    Nonsolvent water should thus not be considered as being ''bound,'' although a small part of it may actually be more or less bound to certain groups, especially charged groups and dipoles. This concerns, for proteins, about 0.1–0.2 g per g of protein. Neither should bound water be confused with held water or imbibition water, which is mechanically entrapped. For instance, casein micelles may contain as much as 3 g held water per g casein (see Section 3.2), but this water diffuses freely in and out of the micelles. If milk is renneted and forms

a gel built of paracasein (see Section 21.3), all of the water is entrapped (held) in the gel, i.e., about 40 g of water per g paracasein, whereas nonsolvent water for lactose still is about 0.55 g/g.

9.1.2 Water Activity

If the water content of a product decreases, its water activity (a_w) also decreases. Water activity is expressed as a fraction. In pure water $a_w = 1$; in a system without water, $a_w = 0$. For ideal solutions, $a_w = m_w$, where m_w is the mole fraction of water in the solution, i.e.:

$$m_w = \frac{\text{moles of water}}{\text{moles of water } + \text{ moles of solutes}} \qquad (9.2)$$

The following explain why most of the time $a_w < m_w$.

    a. Dissociation of compounds, e.g., salts, causes a higher molar concentration of solute species.

    b. The effective concentration of solutes is considerably increased if their occupied volume is large. This is an essential factor when substances of a high molar mass are present.

    c. Part of the water may not be available as a solvent (see Section 9.1.1).

All together, it means that in milk products the relationship deviates from $a_w = m_w$, especially when the milk is highly concentrated.

    The water activity of a product can be measured because a_w equals the relative humidity of air in equilibrium with the product. Accordingly, it can be determined by establishing the relative humidity at which the product neither absorbs nor releases water. The following are some examples of a_w values of milk products.

Milk	0.993
Evaporated milk	0.986
Ice cream mix	0.97
Sweetened condensed milk	0.83
Skim milk powder, 4.5% water	0.2
Skim milk powder, 3% water	0.1
Skim milk powder, 1.5% water	0.02
Cheese	0.94–0.98

Any product has its characteristic relation between a_w and water content for every temperature. Such a relation is called a water vapor pressure isotherm or *sorption isotherm.* An example is given in Figure 9.3a. At increasing temperature and constant water content, a_w increases. The a_w of most liquid milk products appears to be fairly high.
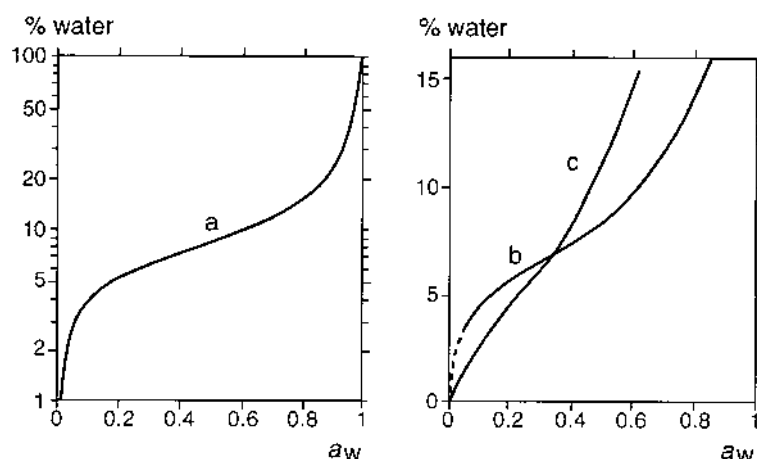
FIGURE 9.3 Water sorption isotherms of concentrated skim milk. (a) Approximate isotherm. (b) Desorption isotherm of skim milk powder with lactose in the crystalline state. (c) Resorption isotherm of skim milk powder with lactose in the amorphous state.

Often, sorption isotherms show hysteresis, i.e., it makes a difference whether the curves are obtained by successively decreasing (desorption) or increasing a_w (resorption). This is because equilibrium is not reached, certainly at very low water contents. It is very difficult to remove the last water in a product [except at high temperature, where other changes (reactions) will occur also]. This implies that, essentially, the water activity involved is unknown because a_w refers to a condition of equilibrium.

In milk, there is a complication caused by lactose. If milk is dried slowly, α-lactose hydrate will crystallize. The water of crystallization present is very difficult to remove and, hence, it does not contribute to the a_w value estimated. This implies that the point 0:0 of the sorption isotherm is not covered (Fig. 9.3b). However, spray drying of milk often yields a powder with amorphous lactose. At similar water contents (i.e., water inclusive of water of crystallization), the latter powder results in a higher a_w (Fig. 9.3c). This is because in the spray-dried powder almost all of the water is available as a solvent. If the powder takes up water, its a_w increases less rapidly and the isotherms b and c (Fig. 9.3) intersect. Eventually, α-lactose hydrate crystallizes. Because of this, at constant a_w (=air humidity) powder c will then lose water. Incidentally, in most of the methods applied for determining water content in dried milk and dried whey, the greater part of the water of crystallization is included in the ''dry matter.''

9.1.3 Changes Caused by Concentrating

Apart from the increase of most of the solute concentrations, removal of water from milk causes numerous changes in properties, which often are approximately proportional to Q^*. The changes also depend on other conditions, such as heat treatment and homogenization before concentrating. Some important changes in properties are as follows:

a. The *water activity* decreases. Examples are given in Figure 9.3.
b. The *hygroscopicity* increases. Usually, a (dry) product is called hygroscopic if a small increase of a_w causes a considerable increase in water content. Obviously, this mainly concerns milk powder with a very low water content (Fig. 9.3).
c. The *salt equilibria* change. The Ca^{2+} activity is increased only slightly because calcium phosphate, which is saturated in milk, turns into an undissolved state (see Section 2.2.4). Because of the latter change, the pH decreases by about 0.3 and 0.5 unit for $Q = 2$ and $Q = 3$, respectively. For $Q = 2.5$, the fraction of Ca that is in solution decreases from about 0.4 to 0.3.
d. The *conformation of proteins* changes because ionic strength (hence, thickness of the electrical double layer), pH, and other salt equilibria all change. If milk is highly concentrated, the solvent quality decreases also. All in all, the tendency of the protein molecules to associate and to attain a compact conformation is increased. Coalescence of casein micelles causes them to increase in size. The increase is smaller if the milk has been intensely preheated, presumably because β-lactoglobulin and other serum proteins have become associated with casein.
e. Several *physicochemical properties* change. Osmotic pressure, freezing point depression, boiling point elevation, electrical conductivity, density, and refractive index all increase, and heat conductivity decreases.
f. *Rheological properties* are affected. The viscosity increases (Fig. 3.20); the liquid becomes non-Newtonian (viscoelastic and shear rate thinning) and finally, solid-like (say, at $Q > 9$ for skim milk). This is all highly dependent on temperature.
g. *Diffusion coefficients* decrease. At low water content the effect is very strong. The diffusion coefficient of water decreases from approximately 10^{-9} m$^2 \cdot$ s^{-1} in milk to 10^{-16} m$^2 \cdot$ s^{-1} in skim milk powder with a small percentage of water.

9.1.4 Reaction Rates

The changes in properties as caused by increasing Q^* may affect the rates of chemical reactions and of physical changes to a considerable extent. In most

cases, a reduction in rate occurs (Fig. 9.4), and often it is specifically for that reason that concentrated products, especially dried ones, are made.

Usually, the main cause of the slower reaction rate after considerable concentration is the decreased diffusion coefficient. Clearly, reacting molecules must collide before they can react, and the probability of collision is proportional to the diffusion coefficient; if diffusivity is sufficiently small, it becomes rate limiting for bimolecular reactions. The slower rate of physical processes such as crystallization is also ascribed to a decreased diffusivity. The larger the molecules, the stronger is the decrease of the diffusion coefficient; in milk powder with a low water content the effective diffusion coefficient may be zero. Most of the powder material, then, is in a glassy state.

It is fairly customary to plot reaction rates against water activity. This practice suggests a_w to be the rate-determining factor but often that is not true. If water is a reactant (e.g., as in hydrolysis), the reaction rate depends also on the water activity itself. However, the reaction rate decreases by a factor of 2 when going from a_w of 1 to a_w of 0.5, whereas the diffusion coefficient then may decrease by a factor of 10^3.

Because of an increase in the concentration of reactants, the rate of bimolecular reactions at first increases due to removal of water. On further increase of
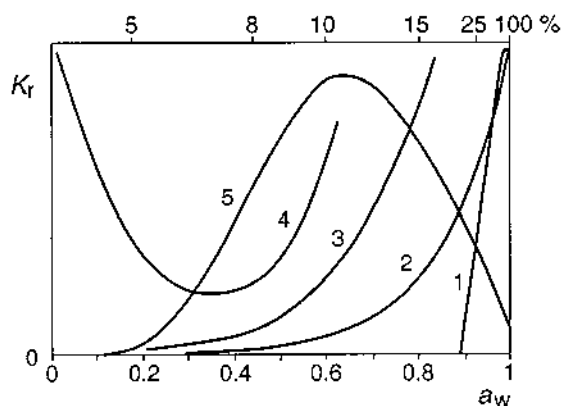
FIGURE 9.4 Relative reaction rate (K_r) of various reactions as a function of the water activity (a_w) of (concentrated) skim milk (powder). The upper abscissa scale gives the approximate water content (% w/w). 1, Growth of *Staphylococcus aureus*; 2, oxidative degradation of ascorbic acid; 3, enzyme action (e.g., lipase); 4, lipid autoxidation; 5, Maillard reaction (nonenzymatic browning). Only meant to illustrate trends. From P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

Q^*, the reaction rate often decreases again; this decrease would be caused by reduced diffusivity. A good example is the Maillard reaction (Fig. 9.4, curve 5). The irreversible loss of solubility of milk protein in milk powders and the rate of gelation of concentrated milks show a trend similar to curve 5. On removal of water from milk, it thus is advisable to pass the level of approximately 10% water in the product as quickly as possible.

Autoxidation of lipids follows quite a different pattern (Fig. 9.4, curve 4). The reaction rate is high for low a_w. Possible causes are that water lowers the lifetime of free radicals, slows down the decomposition of hydroperoxides, and lowers the catalytic activity of metal ions, such as Cu^{2+}.

Heat denaturation of globular proteins and, consequently, inactivation of enzymes and killing of microorganisms (see Section 6.3) greatly depend on water content. An example is given in Figure 9.5 (alkaline phosphatase). Both $\Delta H^\ddagger$ (activation enthalpy) and $\Delta S^\ddagger$ (activation entropy) for denaturation usually decrease with decreasing water content. This implies that the dependence of denaturation or inactivation rate on temperature also decreases with decreasing water content. It may also occur that removal of water increases the concentration of a reactant or catalyst for heat inactivation; this is presumably the case for chymosin in whey (Fig. 9.5), since at, say, 40% dry matter a_w and diffusivity are not greatly lowered.

Growth and metabolic action of microorganisms closely depend on a_w. Many bacteria have an optimal a_w for growth of about 0.99 and a minimal a_w
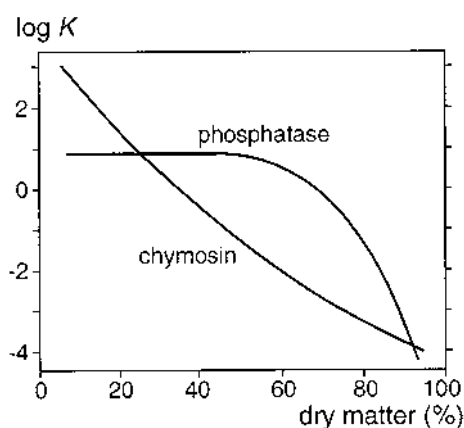
FIGURE 9.5 Reaction constants (K, in s^{-1}) for inactivation of alkaline phosphatase in concentrated skim milk and of chymosin in concentrated whey at 80°C, as a function of water content. Approximate examples. After A.L.H. Daemen, *Neth. Milk Dairy J.* **35** (1981) 133.

of 0.96–0.90. An example is given in Figure 9.4, curve 1. Small differences in a_w thus can have considerable effect on growth rate. Most yeasts have a minimal a_w for growth of 0.91–0.87, osmophilic yeasts of 0.65–0.60, most moulds 0.87–0.80, xerophilic moulds 0.75–0.65, halophilic bacteria 0.80–0.75. The direct relationship between a_w and the osmotic pressure Π (this relationship is roughly $\Pi = 14 \times 10^7(1 - a_w)$ in Pa) is usually held responsible for the influence of a_w on the growth of microorganisms. An excessive increase of Π will cause dehydration of the organism, and the organism thus will die. The effects may differ, however, depending on which substances cause the low a_w. A high concentration of salt retards the growth of osmophilic yeasts, but a high concentration of sugar does not. Halophilic bacteria, in turn, are salt-tolerant. In other words, growth of microorganisms is inhibited not so much by a low a_w as by a high concentration of specific substances. Of course, when water is removed, inhibiting and stimulatory substances are also concentrated.

9.2 EVAPORATING

Evaporation of products like milk, skim milk, and whey is applied:

    a. To make such concentrated products as evaporated milk, sweetened condensed milk, and concentrated yogurt

    b. As a process step in the manufacture of dry milk products, considering that the removal of water by evaporation requires far less energy than by drying (see Table 9.2)

TABLE 9.2 Heat of Evaporation of Water and Approximate Energy Requirement in Some Processes to Remove Water[a]

Heat of evaporation of water at 100°C	2255
Heat of evaporation of water at 40°C	2405
Sorption heat for evaporation of water from skim milk up to about 60% dry matter	~5
Evaporation, 3 stages	~800[b] (0.35 kg steam per kg water)
Evaporation, 6 stages, with thermal vapor compression	~230[b] (0.1 kg steam)
Evaporation, 1 stage, with mechanical vapor compression	~115 (0.05 kg steam)
Roller drying	~2500[b] (1.1 kg steam)
Spray drying	~4500[b] (2.0 kg steam)
Reverse osmosis	20–35

[a] All data are in kJ or kg steam per kg water removed.
[b] Excluding mechanical energy (pumps, etc.).

    c.   To produce lactose (α-lactose hydrate) from whey by means of crystallization

Important aspects of the evaporation of milk and milk products are discussed in Sections 6.2 and 9.1. Alternatives to evaporation are reverse osmosis (Section 9.4.2) and freeze concentration.

    Evaporation is always done under reduced pressure, primarily to allow boiling at a lower temperature and thus prevent damage due to heating. Figure 9.6 shows the vapor pressure as a function of temperature, i.e., the relation between pressure and boiling temperature of pure water. This relationship disregards elevation of the boiling point due to dissolved substances, which is, however, fairly small: for milk 0.17 K, for evaporated skim milk up to about 2 K, and for evaporated whey and sweetened condensed milk up to slightly more than 3 K. Moreover, evaporation under vacuum facilitates evaporation in several stages, which results in a considerable saving in energy and in cooling water for the condenser. The water vapor, formed by the evaporation, is then used as ''steam'' to cause boiling of the liquid in the next effect. Figure 9.7 gives a diagrammatic representation of a multiple-effect evaporator.

    In principle, nearly all evaporation energy can be recovered (except for a small amount of sorption heat, essentially the additional energy needed to remove
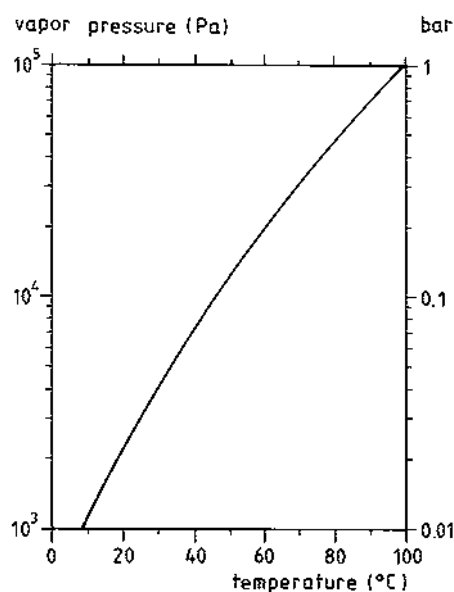
FIGURE 9.6 Vapor pressure of water as a function of temperature.
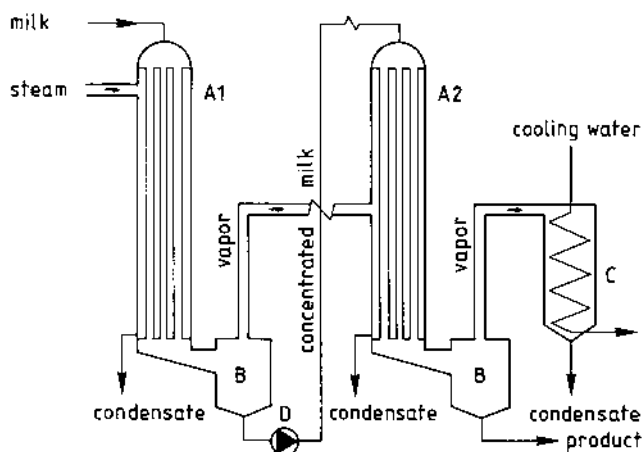
FIGURE 9.7 Principle of a multiple-effect falling film evaporator for milk. Highly simplified. The number of effects may vary from 3 to 7. (A) Evaporation unit (''effect''). (B) Vapor separator. (C) Condenser. (D) Pump. Temperatures in A2 are lower than in A1.

water against an increase in osmotic pressure) by concentrating in a great number of effects and applying thermal vapor compression (this means increasing temperature and pressure of the vapor by injecting some steam in it). There is, however, a limit. Radiation causes heat loss to the surroundings, the vapor leaving the last effect implies heat loss, etc. Furthermore, the temperature difference between condensing vapor and evaporating liquid in the separate effects (ΔT) decreases as the number of effects increases. Too small a ΔT should be avoided because otherwise a very large heating surface would be needed and, hence, a large and expensive plant, losing much heat by radiation and imposing high cleaning expenses. Moreover, the boiling point elevation lowers ΔT in each effect: the temperature of the boiling liquid is higher than that of the vapor and the latter is utilized to bring to boiling the liquid in the next effect. The temperature differences between the effects can, of course, be increased by increasing the temperature in the first effect or by lowering that in the last. The former measure soon leads to undesirable changes in the milk; the maximum temperature allowed obviously depends on the kind of product that is to be made. Lowering the temperature in the last effect causes the liquid to become highly viscous, which leads to poor heat transfer (see Section 6.5). Figure 9.8 gives an example of the course of the evaporation under practical conditions.

    Some of the problems mentioned can be overcome by application of a large single-effect evaporator, in which the vapor is mechanically compressed and re-
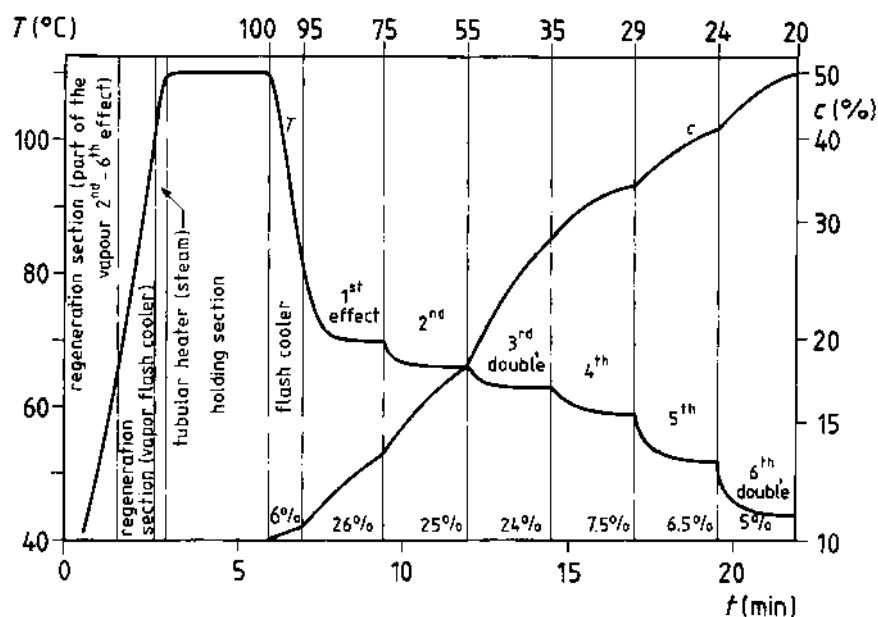
FIGURE 9.8 Example of the course of temperature (*T*) and dry matter content (*c*) of skim milk as a function of time (*t*) in a six-effect evaporator with preheating. The scale for the dry matter content is logarithmic. The milk is preheated with heat exchangers that successively use exhaust vapor of the evaporators, vapor of the cooler, and live steam. Furthermore, a part of the vapor of the third effect is compressed with steam and led to the first effect. For the rest, vapor and concentrate are cocurrent. A part of the water is evaporated in a vacuum cooler by flash evaporation. The percentages mentioned indicate the proportion of the water evaporated in the effect concerned (89% of all water being eventually removed). The numbers on top represent the residual liquid (in mass percent) after the various effects. Data from Stork-Friesland.

circulated in the evaporating unit to be used as steam. In this manner, the boiling temperature is constant and can be as high as the product allows, thereby enhancing heat transfer rate. The total energy consumption can be as low as 0.05 kg steam per kg of water evaporated. At the time of writing, there was little experience with this type of evaporator, but its use holds much promise.

The above-mentioned elevation of the boiling point can be ascribed to the presence of dissolved substances and to the hydrostatic pressure. At an air pressure of about 10^4 Pa (boiling point about 50°C), the hydrostatic pressure of a 1-m-high column of evaporated milk will elevate the boiling point by about 15 K.

Because of this, falling film evaporators are commonly applied when the product is evaporated in a large number of effects because these cause virtually no hydrostatic pressure. In such an evaporator, the milk flows as a thin film along the inside of a number of pipes that are heated from the outside by condensing steam. This does not mean that there is no pressure loss; friction causes some decrease of pressure, hence decrease of ΔT, over the pipe length, especially if the pipes are narrow.

When manufacturing powder, the amount of water removed in the evaporator should be as large as possible. The limit is set by the high viscosity of the concentrate (see also Fig. 9.9). A high viscosity retards the flow rate near the heating surface and thereby the heat transfer. In a falling film evaporator, in which the flow rate is fairly high, the viscosity should not exceed about 0.1 Pa · s. At a low temperature, highly concentrated milk and skim milk would exceed this limit. Therefore, partial countercurrent flow is sometimes applied in multiple-effect evaporators. The concentrate passes through the last effects in the reverse order, the highest concentrated milk being evaporated not at the lowest temperature but at a somewhat higher one, which leads to a lower viscosity. Obviously, this is only feasible if vapor compression is applied. When making sweetened condensed milk, a viscosity of over 0.1 Pa · s is eventually reached. In the last effect a fairly large conventional rising film evaporator (with natural circulation) is then often applied, in which the milk is partially recirculated to prevent the heating surface from partly running dry.

The viscosity of the concentrate thus is an important parameter in the evaporating process (as it is in the spray drying process, where it affects the droplet size in the spray; see Section 9.3). Several factors affect the viscosity; see, for example, Figure 9.9. The viscosity increases more than proportionally with the dry matter content. The relatively strong increase upon increasing dry matter contents is explained by the particle volume fraction in the liquid already being very high [see Equation (3.5) for the case that φ approximates φ_{max}]. The evaporating milk is markedly shear thinning, and thus its viscosity is an apparent one, η'. This implies that at a shear rate of, say, $100 \ s^{-1}$, η' is about twice the value at $2000 \ s^{-1}$. Preheating of the milk increases η' considerably if the dry matter content is high. This may tentatively be explained by the serum proteins greatly increasing in voluminosity due to denaturation. The influence of the temperature on η' is hard to estimate because η' rapidly increases with time at high temperature and high dry matter content, a process called age thickening. Eventually this leads to gelation (see Fig. 9.9C).

The degree of concentration is usually checked by means of the density ρ or the refractive index n. These parameters can be determined continuously in the concentrate flow. This enables automatic control of the evaporating process by adjusting the steam or the milk supply. This is far from easy, given the prolonged holdup time and the great number of process steps.
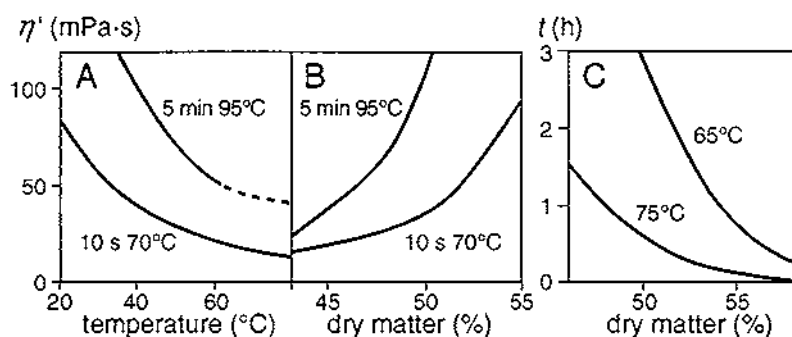
FIGURE 9.9 Apparent viscosity (η′) of skim milk concentrate of various dry matter content. (A) Effect of temperature, parameter is preheating, 48% dry matter. (B) Effect of concentration, same parameter, measuring temperature 50°C. (C) Time needed (*t*) to cause gelation of the concentrate at two temperatures as a function of concentration. Approximate results after T.H.M. Snoeren et al., *Neth. Milk Dairy J.* **38** (1984) 43–53.

Product properties to be considered include the following:

a. Age thickening at high concentration and at high temperature.
b. Highly evaporated milk is susceptible to Maillard reactions (Fig. 9.4).
c. Fouling occurs readily if the product is highly concentrated, the temperature is high, the temperature difference across the wall is high, and the flow rate of the liquid is slow. Preheating may significantly diminish fouling at high temperature. The construction of the equipment greatly affects the rate of fouling and the ease of cleaning. Cleaning expenses increase with the heating area of the equipment, hence with the number of effects in a multiple-stage evaporator.
d. Bacteria can grow at fairly high temperatures, which mostly means in the last effects. True thermophilic bacteria are involved like *Bacillus stearothermophilus*, which may even survive sterilization. This implies that processing must be done hygienically and that the plant must be cleaned and disinfected after not more than 20 hours of continuous operation. The spread in holdup time is also of importance (see Section 17.3).
e. Foaming mainly occurs with skim milk, at a fairly low temperature. The machinery should be adapted; a falling film evaporator presents few problems.
f. Disruption of fat globules especially occurs in falling film evaporators. For instance, d_{vs} may decrease from 3.8 to 2.4 μm by evaporating milk

up to 50% dry matter. Usually, this is not a problem because the milk
is homogenized anyway. In some evaporators the fat globules can co-
alesce, which would necessitate homogenization.

g.   Premature crystallization of lactose, causing rapid fouling of the equip-
ment, may occur especially in highly concentrated whey at a low tem-
perature. Figure 9.10 gives an idea about the rate at which this may
occur.

It should be realized that different products allow different degrees of evaporation
and that the same concentration factor affects the concentration of dissolved con-
stituents differently. Table 9.3 gives some examples: Lactose will not crystallize
in highly concentrated milk, whereas it may do so readily in highly concentrated
skim milk. Concentrated whey with its high Q^* may show considerable fouling of
the evaporator equipment, due to supersaturated salts precipitating on the heating
surface. This drawback can largely be overcome by keeping the partly evaporated
whey outside the equipment for some time (say, 2 hours) before it is further
concentrated. The salts then are allowed to crystallize in the bulk and lactose
crystallizes at the same time.
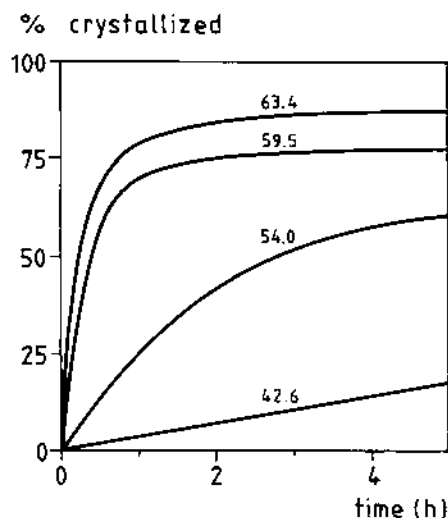
FIGURE 9.10   Crystallization of lactose in concentrated whey (parameter is %
dry matter) as a function of the time after cooling to 20°C. In the range of 15–
40°C, the crystallization rate depends little on the temperature. After results
by K. Roetman (Ph.D. Thesis, Wageningen Agricultural University, 1982).

TABLE 9.3 Approximate Composition of Liquids Evaporated up to the Maximum Degree Possible

Liquid	% Dry matter	Q^a	$Q*$	Saturation of lactose[b]
Milk	50	4	7	1.05
Skim milk	55	6	12	1.85
Sweet whey	64	9.5	25	3.77

[a] Q = concentration factor, $Q*$ = concentration relative to the water content (see Section 9.1).
[b] At 40°C and on the assumption that evaporation does not alter the activity coefficient of lactose. Presumably, the coefficient increases, thereby increasing the actual supersaturation (especially in sweet whey).

9.3 DRYING

9.3.1 Objectives

Drying is usually applied to make a durable product that is easy to handle and, after reconstitution with water, is very similar in properties to the original material. Drying is applied to products like milk, skim milk, whey, cream, ice cream mix, protein concentrates, infant foods, all of which have a high water content. Removal of water is expensive, especially with respect to energy (see Table 9.2). Furthermore, driers are expensive. Therefore, the material is often concentrated to a fairly low water content by evaporation (Section 9.2) or by reverse osmosis (Section 9.4) before drying.

The main technological problem is to prevent the drying product from undergoing undesirable changes. The rate of many reactions greatly depends on the water content; an example is given in Figure 9.11 (see Section 9.1). It mainly concerns reactions that render protein insoluble, since these strongly depend on temperature: $Q_{10} \approx 4.5$ (see also Fig. 9.9C). At 80°C, about half of the protein present in concentrated skim milk with 13% water becomes insoluble in 10 s. Thus it is advantageous to pass the interval from, say, 20% to 8% water rapidly and at a moderate temperature. However, the effective diffusion coefficient of water and, consequently, the drying rate significantly decrease with decreasing water content and with decreasing temperature (Fig. 9.12). The following calculation may be illustrative. Consider the drying of concentrated skim milk in a thin layer of thickness $x = 1$ mm. Figure 9.12 shows that the diffusion coefficient (D) of water at 70°C is on average about 2×10^{-11} m$^2 \cdot$ s^{-1} when the water content is to be halved from 20% to 10%. According to the relation $x^2 = Dt_{0.5}$,

relative reaction rate
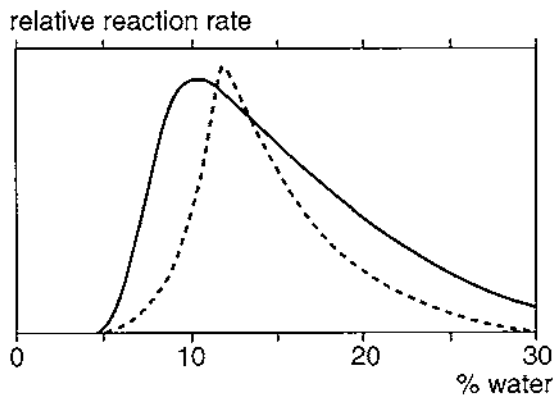
FIGURE 9.11   Rate of Maillard reactions (—) and of protein becoming insoluble (---) in concentrated skim milk at high temperature (say, 80°C) as a function of water content. The curve for insoluble protein depends on several conditions such as preheating. Approximate examples.

effective diffusion coefficient (m²·s⁻¹)
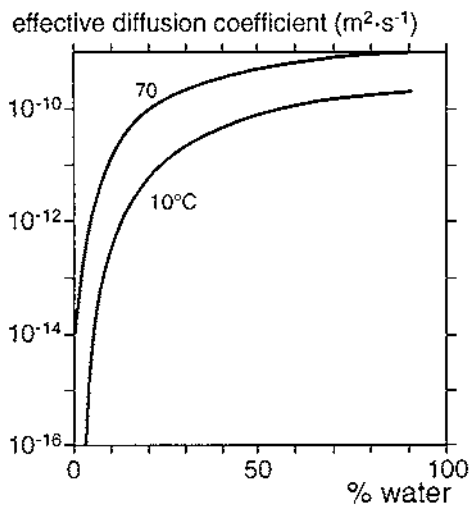
FIGURE 9.12   Effective diffusion coefficient of water in drying skim milk as a function of water content, at two temperatures. After P.J.A.M. Kerkhof (Ph.D. Thesis, Eindhoven Technical University, 1975).

the time required for this would be 5×10^4 s, i.e., about 14 hours. The liquid therefore will have to be atomized very finely if its drying is to be fast. Alternatively, the drying can be carried out at low temperature, but that usually takes a long time.

9.3.2 Drying Methods

There are several methods for drying liquids. The dairy manufacturer uses only a few of them.

9.3.2.1 Drum Drying

A thin film of milk, skim milk, etc., is dried on a large rotary metal cylinder or drum that is steam-heated internally. Often, two drums are set up side by side. The water evaporates within a few seconds. The dried film is scraped off from the drum by means of a steel knife, collected, and ground. Considerable product damage due to heating occurs, mainly because scraping off is always imperfect and, accordingly, a part of the milk is repeatedly wetted and dried. The quality of the powder can be improved by using a vacuum roller drier, in which the milk is dried at a lower temperature; but this method is expensive. Nowadays the roller drying process is little used.

9.3.2.2 Foam Drying

Under pressure, air or nitrogen is injected into the concentrate, and the mixture obtained is heated in a vacuum. Many gas cells are formed in the concentrate, which soon turns into a spongy mass that can subsequently be dried fairly quickly. The process can be carried out batchwise (concentrate in shallow trays) or continuously on a conveyer belt. The dried cake is ground to a voluminous, easily soluble powder. The powder quality can be excellent due to the low drying temperature applied. The process is expensive and is only applied for some composite products like infant formulas. An advantage of the method is that it can be applied to inhomogeneous products.

9.3.2.3 Freeze Drying

A thin layer of the liquid is frozen, whereupon the ice is sublimated under a high vacuum. A voluminous cake is left (the space of the ice crystals is now occupied by holes) and is subsequently ground. A batch processing or a continuous operation in a high-vacuum belt drier can be applied. The method is expensive. Damage due to heating does not occur, but that also holds for spray drying if skillfully performed. The drawback is that nearly all of the fat globules coalesce, which causes freeze-dried whole milk powder to show segregation after its reconstitution. Freeze drying is suitable for processing in small quantities and is applied in the drying of lactic starters, etc.

9.3.2.4 Spray Drying

This is the common method. There are several variants, but the following are essential process steps that are always involved (see Fig. 9.13).

a. *Air heating*. To achieve this, the air is passed around bundles of steam pipes (steam pressure is 9 atm to reach air temperatures up to 175°C) or the air passes a wall heated by gas jets to reach at most 260°C. Nowadays, the latter is the common method. A more economic use of heat is made by direct combustion of gas in the drying air, but this process releases nitrogen oxides that would contaminate the powder. The air leaving the drier at, say, 100°C is sometimes used to warm up the fresh air in a heat exchanger.

b. *Atomizing the concentrate in the air* to such small droplets as to dry very quickly, with either a spinning disk or a pressure nozzle. Often, the liquid is first heated to a suitable temperature.

c. *Mixing hot air and atomized liquid*. Drying occurs correspondingly. Air and liquid usually enter the drying chamber cocurrently and are mixed so intensely that the air cools very rapidly. Consequently, the larger part of the drying process occurs at temperatures not much over the outlet temperature. In other words, something close to perfect mix-
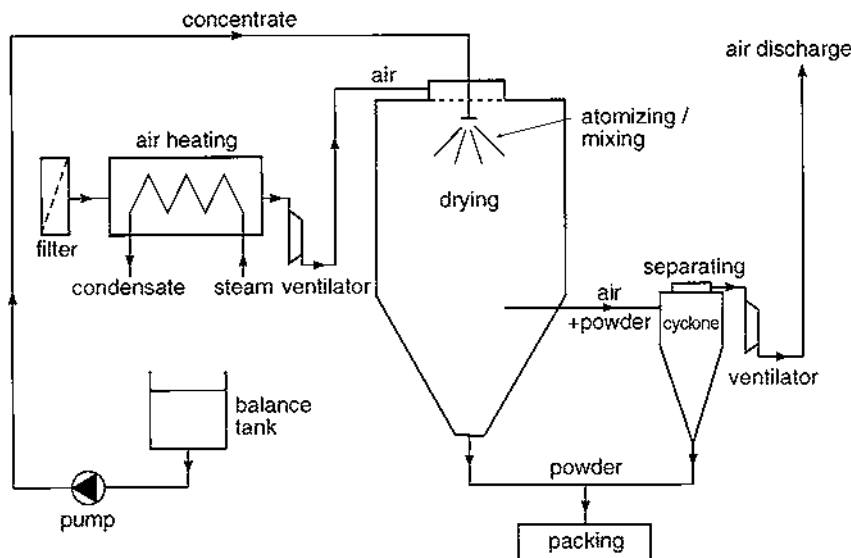
FIGURE 9.13 Simplified diagram of an example of the spray-drying process.

ing occurs. The shape of the drying chamber is of great importance: the larger the chamber (at a given capacity), the more expensive; the smaller, the greater the risk of incompletely dried droplets touching the wall and fouling it. Furthermore, too intense heating of part of the drying droplets should be prevented. Obviously, the mixing should comply with strict requirements. However, drying chambers generally are designed on the basis of experience and the mixing is not quite as desired.

d.   *Separating powder and consumed drying air*. Cyclones are commonly used; essentially, the chamber itself also acts as a cyclone. On the one hand, the aim is to accumulate the powder in such a way that it can readily be packed; often, it is necessary to cool a little, e.g., by adding cold air. On the other hand, the amount of powder in the outlet air should be small because it implies loss of yield as well as air pollution. A complicated system of cyclones is often applied. For example, the air released from a powder-separating cyclone is purified in a second cyclone or by means of filters. Sometimes the finest powder is returned to the chamber. Alternatively, the outlet air passes a wet washer, in which it collides with a water film that takes up the residual powder.

Sections 9.3.3 to 9.3.5 cover certain aspects of spray drying in greater detail.

9.3.2.5   Final Drying

In the drying of a liquid several stages can be distinguished, e.g., a stage in which the liquid turns into a more or less solid mass and a stage in which the solid mass obtained decreases further in water content (''final'' drying). In milk products, a solid material is obtained at a water content near 8% (the product obtained is no longer sticky and appears to be dry), whereas a powder with, say, 3% water is desired. Traditionally, one process step included both drying stages, though in freeze drying the temperature must be raised during the final drying to complete it within a reasonable time. In spray drying, advantage is often taken of separating the final drying from the main process. This so-called two-stage drying is discussed in Section 9.3.6.

9.3.3   Atomization

Atomization is aimed at forming droplets fine enough to dry quickly, but not so fine as to escape with the outlet air after having been dried. Moreover, a very fine powder has undesirable properties because it is hard to dissolve; skim milk powder is readily blown away.

Often, atomization is done with a spinning disk at 200–300 revolutions per second. There are several types of disks, but essentially, the liquid falls on a disk

and is flung away at a high speed, e.g., 100 m · s^{-1}. Among the advantages of disk atomization are the following:

a. Flexibility of the manufacturing process is such that the capacity can be varied over a fairly wide range.
b. The disk does not readily become clogged. For example, precrystallized concentrated whey can be atomized.
c. Disk atomization is still practicable at high viscosity; highly evaporated milk can thus be processed.
d. Formation of relatively small droplets takes place.

A drawback is that many vacuoles are formed in the particles (see below); furthermore, the droplets are flung away perpendicularly to the axis of the disk and, accordingly, the chamber has to be wide to prevent the droplets from reaching the wall. Roughly speaking, the distance covered by the droplets in a horizontal radial direction is at least 10^4 times the droplet diameter.

Likewise, there are various types of pressure nozzles. Usually, the liquid is forced through a small opening at high pressure (up to 20 MPa) after it has been given a rotating motion. Advantages of the nozzle are its simple construction, the possibility to adjust the angle of the cone-shaped spray of the atomized liquid (thereby allowing a relatively small diameter of the drier), and a low vacuole content in the powder particles. A drawback is that the capacity is fairly small and that it can hardly be varied. In large driers, therefore, several nozzles must be fitted simultaneously. Moreover, a nozzle wears badly and becomes readily clogged.

9.3.3.1 Droplet Size Distribution

Determination of the size distribution of the formed droplets is difficult. Usually, the produced powder is taken as a basis, but that involves several uncertainties: the droplets often shrink very unevenly; they may contain vacuoles; the powder particles may become agglomerated; none of the methods available for particle size determination is fully reliable. Consequently, results as shown in Figure 9.14A are not quite correct.

In disk atomization, the average droplet diameter roughly follows:

$$d_{vs} \approx \text{constant } (Q\eta/\rho N^2 R)^{0.25} \tag{9.3}$$

where Q is feed capacity (in m^3 · s^{-1}), η is viscosity and ρ is density of the atomized liquid, N is number of revolutions per second of the disk, and R is disk diameter. (Note that the concentrate shows non-Newtonian behavior and thus is characterized by an apparent viscosity that depends on the velocity gradient. During atomizing, the velocity gradients would be high.) The constant closely depends on constructional details of the disk. The average droplet size will be larger at a higher dry matter content and at a lower temperature, since both affect the
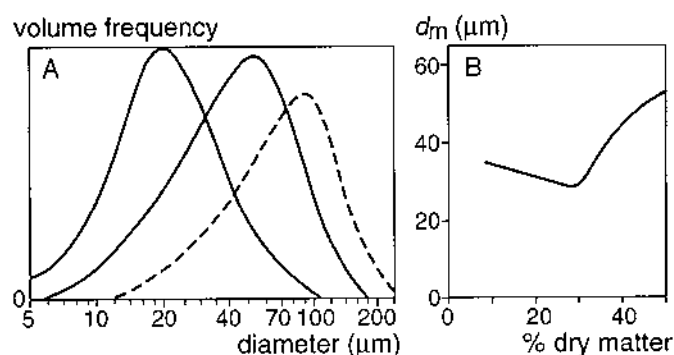
FIGURE 9.14 Particle size of skim milk powder, obtained by applying disk (—) or pressure nozzle (---) atomization. (A) Examples of the volume frequency distribution. (B) Influence of the dry matter content of the concentrate on the median diameter.

viscosity. Near 60°C, d_{vs} is roughly proportional to $T^{-0.33}$ (T in °C); incidentally, at a high temperature η increases rapidly due to age thickening. In Figure 9.14B, the decrease in particle size with increasing dry matter content to about 30% follows from formation of a high vacuole volume in the powder particles if a weakly concentrated milk is atomized (see Section 9.3.3.2).

In nozzle atomization, d_{vs} roughly follows

$$d_{vs} \approx \text{constant } (Q\eta/p)^{0.33} \tag{9.4}$$

where p = pressure in the liquid before the pressure nozzle. The constant depends closely on the construction of the nozzle. p and Q cannot be varied much (otherwise the nozzle does not work at all). At a high η the size distribution becomes fairly wide. Clearly, in disk atomization milk can be more concentrated than in nozzle atomization.

9.3.3.2 Vacuoles

During the atomizing of a liquid, some air is always trapped in the droplets. This generally affects some 10–100 air bubbles per droplet when a disk is used, whereas the number is far less when applying a nozzle, often 0 or 1 air cell per droplet (see also Fig. 9.21). During the drying of the droplets, water vapor enters the air bubbles, causing them to expand; this is because the water vapor can more easily diffuse to the vacuoles than across the external layer of the drying droplets, which has already been concentrated and become more or less rigid. This explains

why the vacuoles are only partly filled with air (see Fig. 9.15A). Raising the drying temperature increasingly expands the vacuoles and enlarges the vacuole volume. (Note that inlet and outlet temperature are both correlated to the drying temperature, with the outlet temperature generally closest; see Section 9.3.5.) Cracks form in the powder particles at high drying temperature. They cause the vacuoles to come into contact with the surrounding air. When the specific volume of such powder particles is determined in air, a low vacuole volume is found, whereas in paraffin oil a high value is observed (Fig. 9.15B); this is because the oil penetrates the vacuoles very slowly, i.e., after many hours. Furthermore, the vacuole volume greatly depends on the dry matter content of the concentrate (Fig. 9.15C). This should largely but not exclusively be ascribed to the influence of the dry matter content on the viscosity. A lower viscosity is part of the reason for a higher vacuole volume at a higher atomizing temperature (which is related to the inlet temperature).

Before as well as during droplet formation, air can be trapped in the droplets, especially when disk atomization is applied. The former mechanism can virtually be excluded by adapting the construction of the disk (Fig. 9.15C). Entrapment of air during droplet formation can largely be overcome by substituting
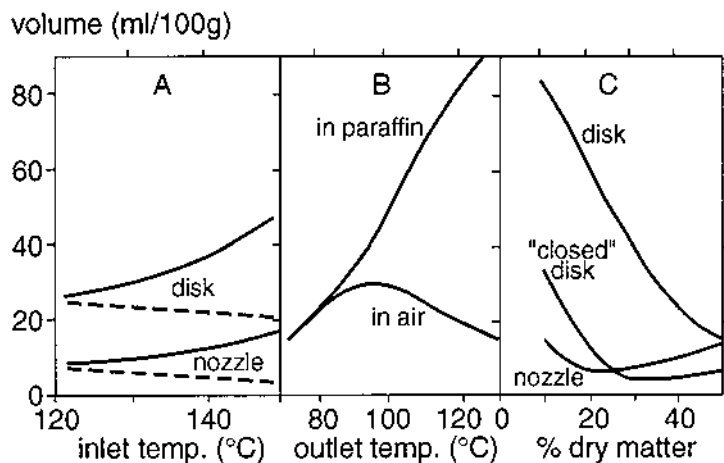
FIGURE 9.15 Volume of vacuoles (——) and of retained air (---) in powder obtained by spray drying evaporated skim milk; determined shortly after drying. (A) Effect of the inlet temperature of the air. (B) Effect of the outlet temperature; disk atomizer. The vacuole volume was determined in paraffin oil or in air (see text). (C) Effect of the dry matter content of the concentrate and of the construction of the atomizer.

the air around the spray nozzle or the disk with steam. The trapped steam bubbles condense so that few if any vacuoles can form. Bathing the atomizer in steam increases the temperature of the liquid during atomization and may thereby cause an increase of the insolubility of the powder.

The importance of vacuole content in terms of properties of the powder is discussed in Chapter 17.

9.3.3.3 Disruption of Fat Globules

This especially occurs during nozzle atomization. It need not be surprising because the applied pressures are comparable to those used in homogenizers.

9.3.4 Change of State of the Drying Air

Properties of moist air can be shown in a state diagram. In analyzing drying processes, a useful diagram is the one according to Mollier. Figure 9.16 gives an example of a partial Mollier diagram (there are several other versions). On the horizontal axis the water content is plotted in kg of water per kg of *dry* air, on the vertical axis the temperature (T) in °C as well as the enthalpy (h) per unit of mass. The lines for T and h are not horizontal. h is defined as the amount of heat needed to bring 1 kg of dry air $+ x$ kg of water from 0°C to T°C; the heating has to include evaporation of the water. h thus is expressed per kg of dry air, but it is the enthalpy of the moist air, including that of the water vapor. By definition, $h = 0$ for dry air of 0°C and for water of 0°C. The diagram has been constructed in such a way that adding water vapor of 0°C to dry air of T°C corresponds to following a horizontal line from the Y axis. Starting from the Y axis, the lines of constant T therefore rise a little since these correspond to adding water vapor of T°C to dry air of T°C; the slope is $1.93T$, where the factor 1.93 is the specific heat of water vapor at constant pressure (in $kJ \cdot kg^{-1} \cdot K^{-1}$). The calibrated scale on the Y axis holds for h as well as for T because $h = T$ (if expressed in $kJ \cdot kg^{-1}$) if $x = 0$, as the specific heat of dry air at constant pressure happens to be precisely $1 \ kJ \cdot kg^{-1} \cdot K^{-1}$. The lines of constant h run parallel with each other and slope down sharply. This implies that at constant T and increasing x, h increases significantly, which ensues from the definition of h, i.e., h includes the heat of evaporation of the water ($2500 \ kJ \cdot kg^{-1}$ at 0°C).

The properties of the air are fully determined by a point in the Mollier diagram (if the atmospheric pressure remains unchanged). Some other kinds of lines can be plotted, e.g., of constant density or constant volume. In Figure 9.16 lines of constant wet-bulb temperature τ are drawn; τ is the temperature of a water surface that shows rapid water evaporation into the air. The τ lines almost follow the lines of constant h, but not precisely. If we add water of 0°C to air, we follow a line of constant h since, according to the definition, $h = 0$ for water of 0°C. (Adding water of 0°C thus is an adiabatic process and the lines of constant
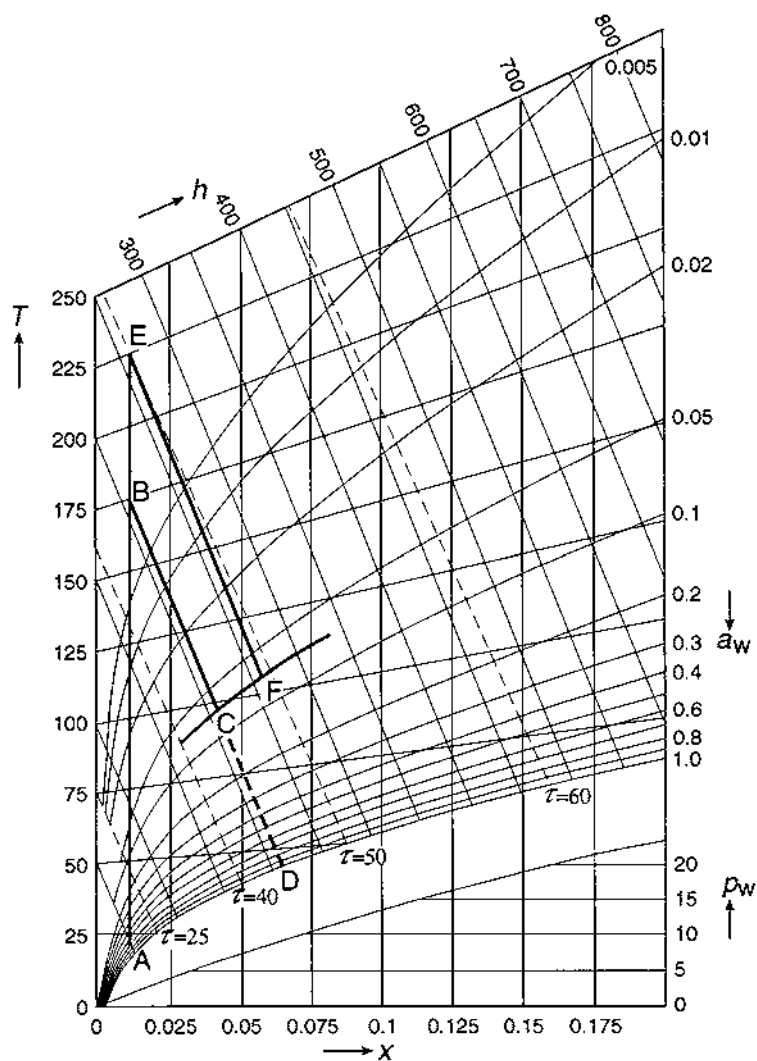
FIGURE 9.16 Partial Mollier diagram of moist air at a pressure of 10^5 Pa (= 1 bar ≈ 0.987 atm). x = water content in kg/kg dry air; T = temperature of the air (°C); h = enthalpy of the moist air in kJ per kg dry air; p_w = absolute water vapor pressure (kPa); a_w = water activity; τ = wet bulb temperature.

h are called adiabatic lines. A process is called adiabatic if there is no heat exchange between the system and its environment.) Lines of constant wet-bulb temperature, however, correspond to the change of state of the air caused by adding water of air temperature. Because of this change, *h* increases somewhat. Knowledge of the quantity τ is important because the driving force for evaporation of water from a drying droplet will be proportional to the temperature difference between the droplet considered and the hot air, hence to $T - \tau$, at least if the water activity of the drying droplet does not deviate too much from 1. Naturally, $T = \tau$ for air saturated with water vapor.

The diagram also gives lines of constant relative humidity or water activity a_w. All of these are curved plots. The importance of the quantity a_w is that it shows the water activity of the drying product after equilibrium between air and product would have been attained. Furthermore, the line for $a_w = 1$ gives the lower limit of the diagram. At the bottom of the figure there is a line that represents the absolute vapor pressure of water (p_w) at constant atmospheric pressure (1 bar). p_w is independent of the temperature (assuming, of course, that $p_w \leq$ the saturation vapor pressure, thus $a_w \leq 1$).

The Mollier diagram can be used in making calculations on the drying process. Consider, for example, air as characterized by point A in Figure 9.16, where $T = 20°C$ and $a_w = 0.7$. The air is heated to 175°C. Since *x* remains constant (0.010 kg · kg^{-1}) we reach point B. a_w decreases sharply, to about 0.002; *h* increases from 45 to 203 kJ · kg^{-1} which means that 158 kJ per kg of dry air $= 134$ kJ · m^{-3} has been supplied. Atomizing a liquid, e.g., concentrated skim milk, will change the conditions of the air along the adiabatic line BD, though not precisely since *h* can change because (a) the temperature of the liquid is $>0°C$; (b) loss of heat to the surroundings occurs; and (c) some sorption heat must be provided during evaporation of water from a solution. In most cases, item c is negligible, and items a and b may roughly neutralize each other.

The temperature to which the drying air may be cooled primarily depends on the corresponding water activity. Ideally, the desorption isotherm of the drying product should be taken as a basis (Fig. 9.17). At 70°C, the curve for $a_w = 0.25$ crosses BD in Figure 9.16. This a_w corresponds to a water content in skim milk powder of, say, 2.5% (Fig. 9.17) which, undoubtedly, is sufficiently low. Consequently, the outlet temperature might be adjusted to 70°C or even somewhat lower. In the above reasoning it has, however, been implicitly assumed that equilibrium between drying air and powder is established, but this is by no means true (Section 9.3.5). In actual practice, drying is commonly continued to yield $a_w \approx 0.07$; for example, this is point C on the line BD (Fig. 9.16), which corresponds to 95°C. Then, if equilibrium would be attained, the water content of the powder should be 1% or even lower, whereas it actually is about 3%.

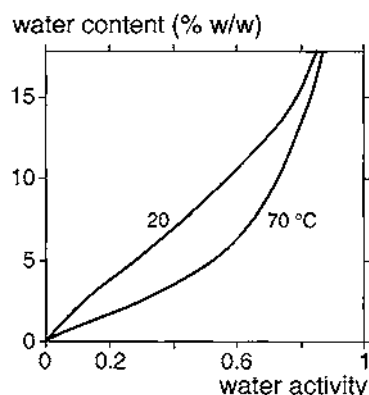The Mollier diagram also shows that in our example the wet-bulb tempera-

water content (% w/w)

FIGURE 9.17 Approximate desorption isotherms of skim milk with noncrystal-lized lactose at 20°C and 70°C.

ture is about 45°C. Initially, the temperature difference between air and droplet may be 175°C − 45°C = 130°C at the most, and is at least about 95°C − 45°C = 50°C. But the actual situation is more complicated; the temperature regime in the drying droplets is discussed in more detail in Section 9.3.5.

In our example, the water content of the air increases from 0.010 (in point B) to 0.041 (in point C) kg per kg of dry air during the drying process. Atomizing a skim milk concentrate with 54% dry matter and drying it to reach 97% dry matter requires [(100 − 54) − (54/97)3]/100(0.041 − 0.010) = 14.3 kg of dry air per kg concentrate. This corresponds to 12.1 m^3 cold air per kg concentrate, since ρ^{20} of air with $a_w = 0.7$ is about 1.18 kg · m^{-3} at a pressure of 1 bar.

The efficiency of the heat expenditure can be expressed and calculated as follows. The heat input per kg of dry air is $(T_i − T_0) c_p$, where T_i is inlet tempera-ture of hot air, T_0 is outside temperature, and c_p is specific heat at constant pres-sure. The heat output is $(T_e − T_i)c_p$, where T_e = outlet temperature of the con-sumed air. Since c_p of air hardly depends on its water content, the efficiency can be defined as $(T_i − T_e)/(T_i − T_0)$. In the present case it would be (175 − 95)/(175 − 20), corresponding to 52%. The amount of heat consumed per amount of evaporated water is $(h_i − h_0)/(x_e − x_i)$ or (203 − 45)/(0.041 − 0.010) = 5097 kJ · kg^{-1}, which roughly corresponds to 2.35 kg steam per kg of evaporated water. The efficiency thus is not high.

Heating the air from 20°C to 225°C, i.e., from A to E in Figure 9.16, implies drying to point F if the same a_w should be reached. This means drying to 106°C, hence to a higher outlet temperature. The average wet-bulb temperature this time is about 50°C. The efficiency amounts to (225 − 106)/(225 − 20), or 58%. In

other words, the higher the inlet temperature, the higher the efficiency. Of course, there is an upper limit with respect to the inlet temperature, partly because of damage to the product caused by heating (Section 9.3.5). Moreover, the powder may catch fire in a drying chamber if it stays for a long time at a high temperature (this concerns powder deposited anywhere in the machinery). Ignition may already occur at 140°C; at 220°C, the time needed for spontaneous ignition is about 5 min.

The Mollier diagram can also be used to study the effects of varying temperature or water content of the outside air, the effect of reuse of air (e.g., by mixing it with fresh air), etc.

It is common practice to control the drying process by adjusting the concentrate supply in such a way that the desired outlet temperature is reached. This can only be achieved within narrow limits, since T_e should be adapted as well if the conditions change drastically. Often, one follows a rule of thumb:

$$\Delta T_e = 0.1 \ \Delta T_i - 5 \ \Delta W + \Delta D \tag{9.5}$$

This relation is to be interpreted as follows:

a. Increasing T_i by 10 K implies that T_e should be increased by about 1 K to maintain a constant water content in the powder (see above).
b. If the percentage of water W in the powder is to be increased by 1 unit, T_e should be lowered by 5 K [the factor of 5 in Equation (9.5) would only apply to skim milk, and even then is approximate].
c. If the percentage of dry matter in the concentrate D is higher by 1 unit, T_e should be increased by about 1 K. This would be due to the increase of the average droplet size with the dry matter content of the concentrate; this increase causes the difference between the water content of the drying droplet and the equilibrium water content of the powder to increase. In other words, to maintain a constant water content of the powder, a lower a_w of the drying air should be reached. The relationship is, however, very approximate.

Currently, more sophisticated methods for controlling the drying process have been developed.

9.3.5 Changes of State of the Drying Droplets

Atomizing pure water in a drying chamber in the usual way causes the water droplets to reach the wet-bulb temperature and to evaporate within 0.1 s at this temperature. The presence of dry matter in the droplets, however, makes an enormous difference. Figure 9.12 shows the diffusion coefficient of water to substantially decrease with increasing dry matter content (e.g., from 10^{-9} to 10^{-13} m$^2 \cdot$ s^{-1}). Accordingly, the drying is significantly slowed down. Moreover, the

water activity of the drying material keeps decreasing, which causes the driving force for drying (roughly proportional to the difference between the a_w of drying air and that of the droplet) to decrease.

In the drying droplet, the thermal diffusivity remains at approximately 10^{-7} $m^2 \cdot s^{-1}$. This implies that in most droplets temperature equalization occurs in less than about 10 ms. In other words, the temperature is virtually the same throughout a droplet, though not at the very beginning of drying.

9.3.5.1 Drying Stages

Initially, the droplet has a very high velocity relative to the drying air. Therefore, there is a first stage where circulation of liquid in the droplet occurs; this circulation greatly enhances transfer of heat and mass. For a droplet of 50 µm diameter this stage lasts, say, 2 ms. In this time, the droplet covers a distance of about 10 cm and loses a small percentage of its water. Its velocity compared to the air decreases to the extent that the formed surface tension gradient of the drop surface arrests internal circulation of liquid. But in the second drying stage the difference in velocity is still great enough to accelerate water transport. To be sure, the transport in the droplet occurs by diffusion, but in the air by convection. After about 25 ms the relative velocity of the droplet has decreased so far that the water transport has become essentially equal to that from a stationary droplet. Relative to the air, the droplet then has covered a distance of a few decimeters and has lost about 30% of its original water. In the third stage, lasting at least a few seconds, the droplet loses the rest of the water by diffusion.

9.3.5.2 Temperature Curve

Assuming for the moment that drying air and droplet remain in equilibrium with each other, the droplet attains the wet-bulb temperature and maintains that temperature until virtually all of the water present has been evaporated. If so, the droplet temperature rises only because the increasing concentration of dry matter eventually leads to a significant elevation of the boiling point (this is equivalent to a decrease in a_w of the drying droplet). The dried droplet finally reaches the outlet temperature of the consumed air. Figure 9.18 shows this to apply reasonably well for a water droplet. But the temperature curve completely changes if dry matter is involved. A droplet of a highly concentrated liquid does not even maintain the wet-bulb temperature for some time. Note in Figure 9.18 that the time needed to arrive at a certain stage in the drying process is always proportional to the square of the initial droplet diameter.

Figure 9.18 refers to drying in stationary air of constant temperature and humidity. Obviously, the actual practice is different. Figure 9.19 gives calculated examples of the course of the drying under conditions similar to those in practice, for air and droplets being truly cocurrent, as well as for perfect mixing. In the usual spray driers we have an intermediate situation, often closer to perfect mix-
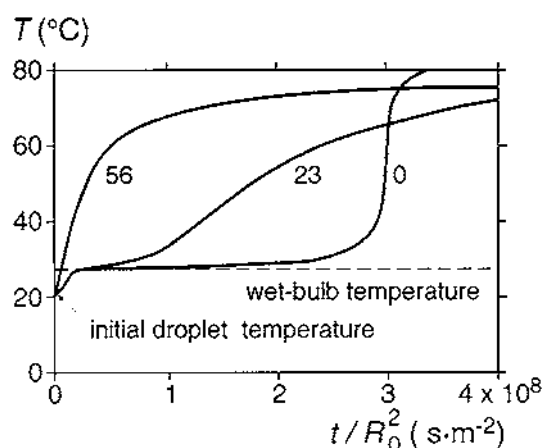
FIGURE 9.18 Temperature (T) of a stationary droplet that enters an excess of dry air of 80°C as a function of the reduced time (t = time after introducing the droplet; R_o = original radius of droplet). The figures near the curves refer to the content (%) of dry matter in the original droplet. Measured by J. van der Lijn (Ph.D. Thesis, Wageningen Agricultural University, 1976).

ing. For perfect mixing, the temperature of the drying droplet is not affected by the inlet temperature of hot air (T_i); for cocurrent drying, it is. In the latter case, the temperature of the droplet can even rise above the outlet temperature (T_e), especially if $T_i \gg T_e$ and if the original water content of the droplet is low. The examples in Figure 9.19 (fairly consistent with actual observations) refer to droplets without vacuoles. If vacuoles are present the drying is faster.

The drying rate of a droplet can be defined as $-d \ln w/dt$, where w = water content of the droplet. It thus is the amount of water removed per unit time, expressed as a fraction of the water left. This rate can be shown (e.g., via calculations from results as shown in Fig. 9.19) to remain roughly constant until a water content of about 15% is reached, and to significantly decrease thereafter.

As already stated, the required drying time greatly depends on the droplet size. The great spread in droplet size within a batch (Fig. 9.14A) causes the drying time to vary widely, roughly by a factor of 200. This spread has another consequence: Figure 9.19 does not apply, at least not for cocurrent drying. The smallest droplets dry quickly. As a result, the hot air cools down, and thereby the larger droplets come into contact with colder air during the longer part of their drying time. Therefore, they have on average a lower drying temperature than the small droplets. In principle, these differences do not occur for perfect mixing.
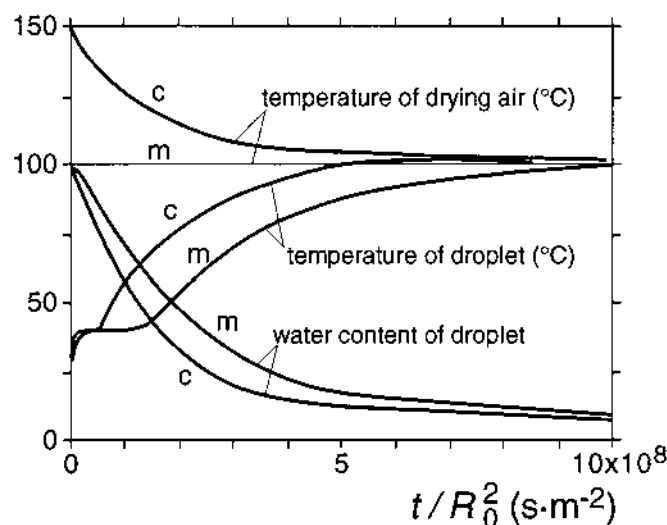
FIGURE 9.19 Variations in temperature of drying air and droplets, and in average water content of the droplets (in % of the original content, i.e., 60%) during drying. Perfect mixing (m), or air and droplets passing cocurrently (c). Inlet temperature 150°C, outlet temperature 100°C. Examples calculated by P.J.A.M. Kerkhof and W.J.A.H. Schoeber (In: A. Spicer, ed.: *Advances in preconcentration and dehydration.* Applied Science Publishing Company, London, 1973).

9.3.5.3 Concentration Gradients

What causes the above-mentioned rapid decrease in drying rate of the droplets after the water content is reduced to 15%? Figure 9.20 shows that a strong concentration gradient forms rapidly during drying. The higher the drying temperature, the stronger the effect. (That explains why stronger gradients occur for cocurrent drying.) Not surprisingly, a dry outer layer, i.e., a kind of rind, is formed; because of this, the water transport is slowed down considerably. The temperature can rise significantly in the dry outer layer because a dried material assumes the air temperature, not the wet-bulb temperature. In other words, the decrease in temperature near the surface of the droplet (caused by consumption of the heat of evaporation) becomes far smaller because the evaporation of water is slower. Because temperature equalization happens very quickly, the whole drying droplet increases in temperature.

Naturally, concentration gradients as shown in Figure 9.20 are not of a
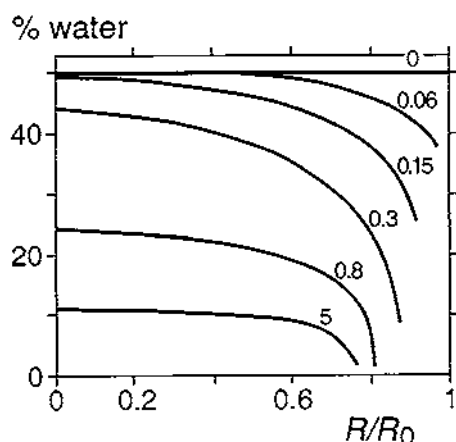
% water

FIGURE 9.20 Water content of a drying droplet as a function of the distance R from the center of the droplet; R_o = radius of original droplet. Parameter is drying time (s) for R_o = 25 μm. Inlet temperature 175°C; outlet temperature 70°C; perfect mixing. Examples calculated by J. van der Lijn (Ph.D. Thesis, Wageningen Agricultural University, 1976).

lasting nature. Let us consider a droplet that has been dried for 5 s; subsequently, it is separated and isolated from the surroundings. The water will become evenly distributed by diffusion and reach about 6% throughout the droplet. Attaining an equilibrium condition takes considerable time because the effective diffusion coefficient D of water is of the order of 10^{-13} m² · s⁻¹ (Fig. 9.12). From $x^2 = Dt_{0.5}$, and considering that the distance x that must be covered approximates 10^{-5} m, we derive that the time needed to halve a concentration difference equals about 10^3 s. All in all, it will take at least 1 h for the concentration gradients to become so small as to be almost negligible.

The relatively dry outer layer of the droplet soon becomes rather firm and thereby causes the droplet to resist further shrinkage. Basically, a pressure below atmospheric develops in the shrinking droplet. The rheological properties of the drying material vary with the kind of material involved and, depending on conditions, various changes may occur. During atomization, air bubbles are trapped in the droplets, and these bubbles can expand in volume to yield large vacuoles (Fig. 9.15). The higher the drying temperature, the stronger the expansion. The particles may also become wrinkled or dented; the effect is stronger as the shrinking is heavier, i.e., as the dry matter content of the feed liquid is lower. Some examples are given in Figure 9.21. Especially at a low water content of the particles, so-called hair cracks may be formed.

A   B   C   D
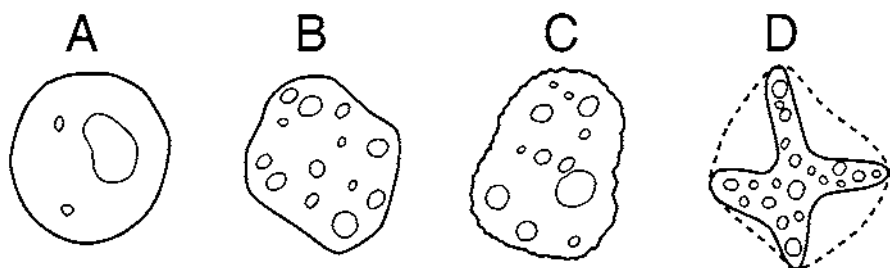
FIGURE 9.21 Cross-sections of powder particles. Highly schematic; only circumference and vacuoles are indicated. Obtained by spray drying of (A) evaporated whole milk, nozzle; (B) evaporated whole milk, disk; (C) evaporated skim milk, disk; (D) skim milk, disk. The broken line shows the outer projection of the particle.

9.3.5.4 Aroma Retention

Besides water, the drying droplets lose other volatile components, including flavor compounds (''aroma''). This is not important for many milk products, but it is for several other foods, e.g., coffee extract and fruit juice. In many cases, however, the loss of flavor compounds is far less than expected, in spite of their volatility. This is because the effective diffusion coefficient of most flavor components in the relatively dry outer layer of the droplet decreases more with decreasing water content than the diffusion coefficient of water does (due to the greater molar mass); the difference can amount to four orders of magnitude. Not surprisingly, the aroma retention (retaining flavor components during drying) increases with droplet size (in larger droplets the outer layer from which the flavor components get lost has a relatively smaller volume) and with drying temperature (at a higher temperature a rind forms more rapidly). Formation of vacuoles diminishes aroma retention, especially if (hair) cracks develop in the particles and the vacuoles come into contact with the surrounding air. In the experiment mentioned in Figure 9.15B, the loss of volatile compounds was almost proportional to the difference between the vacuole volume as determined in paraffin oil and that in air; air can penetrate the cracks in the particles during the measurements, whereas the viscous paraffin oil cannot.

9.3.5.5 Damage Caused by Heating

High drying temperatures can result in undesirable changes in the dried product. Generally, it is only after the powder has been dissolved again that the changes involved are noted. In actual practice, the outlet temperature of the drying air in particular determines the damage due to heating, though the inlet temperature

may also have an effect. The following are possible effects of high drying temperatures:

a. *Inactivation of enzymes*. Often inactivation is considerably slowed down at low water contents (Section 9.1.4), but it may occur before reaching such low water contents as would follow from the results shown in Figure 9.22A. The drying conditions may be adjusted in such a way that any heat inactivation is virtually avoided.
b. *Killing of microorganisms*. (It should be realized that the drying itself, even if carried out at a very low temperature, may reduce the number of living organisms. Such reduction closely depends on the bacterial species present and varies from, say, 10% to 99%.) An example of the total reduction is given in Figure 9.22A. Generally, heat-labile microorganisms will not survive the drying. On the other hand, it is generally not possible to effectively kill all undesirable bacteria by drying.
c. *Denaturation of serum proteins* may be restricted by selecting moderate drying conditions.
d. *Insolubilization of the powder*. A fraction of the protein may be rendered insoluble if the drying temperature is too high at a somewhat low, though not very low, water content (Fig. 9.11). Insolubility is determined by dispersing a quantity of the powder in water under standardized conditions (time, temperature, stirring, etc.) and measuring which part of the dry matter remains undissolved. Figure 9.22B shows an example of insolubility figures. The result greatly depends on other
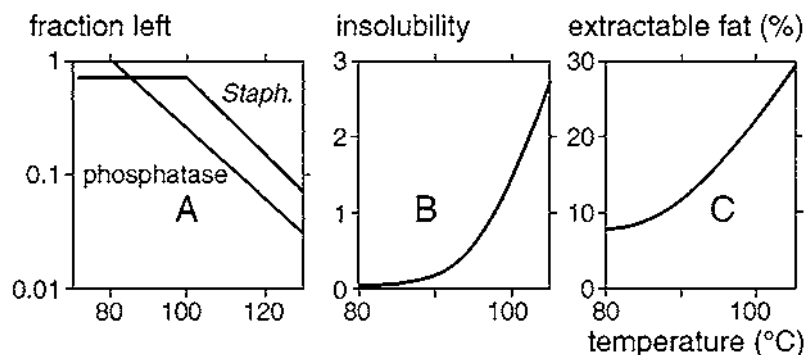
FIGURE 9.22 Examples of the influence of the outlet temperature of the drying air on the inactivation of alkaline phosphatase during drying of the concentrate, the killing of *Staphylococcus* sp., the insolubility index (ADMI) of the powder, and the fat in the milk powder that can be solvent-extracted.

conditions, including pretreatment of concentrate and droplet size. Presumably, increased insolubilization is mainly caused by uneven flow of the air through the drying chamber, as a result of which part of the powder particles may (a) show a relatively long holdup time in the chamber, (b) come back in a zone of high temperature, or (c) be rewetted upon collision with fresh droplets.

   e. *Hair cracks in the powder particles*. A consequence of cracks is that a considerable amount of fat in the powder can be extracted by organic solvents such as petroleum ether and chloroform (Fig. 9.22C). Often, the result of the extraction is called ''free fat'' content, a designation that makes little sense, because by far most of the extracted fat originates from fat globules that are in contact with cracks, vacuoles, or the particle surface and can thereby be removed by the solvent.

9.3.5.6 Particle Size

The size of the drying droplets and of the powder particles is important in relation to the way of manufacture and to the properties of the powder obtained. The larger the particles, the greater the risk of not completely dried droplets touching the wall of the machinery, fouling it and even constituting a fire hazard. The smaller the particles, the more difficult it is to separate them from the drying air.

    The drying time is roughly proportional to R_o^2, where R_o is the radius of the original droplet. This means that a larger droplet maintains a high temperature for a longer time, at a water content at which heat coagulation can occur. Moreover, the water content of a larger particle will be higher when it leaves the drying chamber. This implies that for a larger average droplet size the outlet temperature will be higher (all other conditions being equal) because a smaller amount of heat of evaporation will be taken up from the air. Consequently, when the influence of the droplet size on the powder properties is to be studied, it makes a difference as to whether the outlet temperature, the water content of the powder, or the feed rate of concentrate is kept constant because all of these parameters affect each other. This illustrates the difficulty in interpreting the effects of process variables on powder properties.

    Section 9.3.3 mentions the factors affecting particle size. In actual practice, most of these factors may be fairly constant, with the exception of the viscosity of the concentrate. The viscosity can vary widely (see, e.g., Section 9.2) because (a) it greatly depends on the dry matter content (if it is high); (b) it is much lower at a higher temperature; and (c) during keeping, especially at high temperature and low water content, it rapidly increases due to age thickening. Some examples of the influence of the dry matter content are given in Figure 9.23. Generally, the causality is as follows: high dry matter content → high viscosity → large droplets → high average drying temperature → increased heat damage. Other factors play a role as well. An example is the greater vacuole volume at low
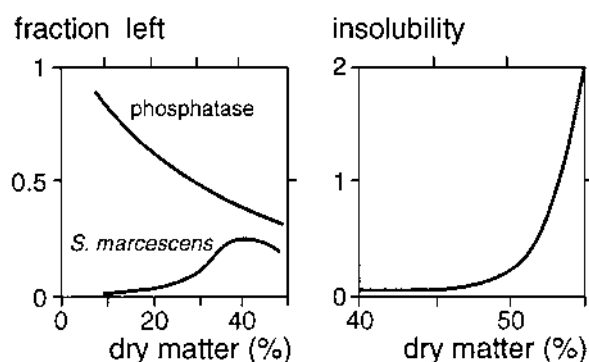
FIGURE 9.23 Inactivation of alkaline phosphatase during drying of a concentrate, killing of a heat-sensitive bacterium (*Serratia marcescens*), and insolubility index of skim milk powder as a function of the dry matter content in the atomized concentrate. Approximate examples.

viscosity of the concentrate (Fig. 9.15C), which enhances drying rate. Figure 9.5 shows that the heat inactivation of the enzyme alkaline phosphatase is slowed down at high dry matter contents. To be sure, this effect only occurs above 50% dry matter. Figure 9.23 shows that the killing of *Serratia marcescens* diminishes at first when the dry matter content increases; this is because the bacterium itself is less temperature-sensitive at a lower water content. It is only after still higher dry matter contents have been reached that the effect of the higher average drying temperature prevails.

9.3.6 Two-Stage Drying

As stated above, spray drying is relatively expensive, e.g., with respect to energy; furthermore, the capital outlay for driers is high. A better efficiency can in principle be achieved by increasing the concentration factor of the milk before atomization and by applying a higher air inlet temperature, but these measures can readily lead to heat damage of the product. Alternatively, the powder can be separated from the air before it is completely dry, while additional drying occurs outside the drying chamber. In this way, the outlet temperature of the air can be lower, allowing the inlet temperature of the air to be higher without increased heat damage occurring. Moreover, a larger quantity of concentrate can be dried per unit time.

The powder may be discharged after it has become so dry as to have lost its stickiness. The problem of stickiness is less than expected because of the concentration gradient formed in the powder particles. Consider, for example, the curve for 0.8 s in Figure 9.20. In the center of the particle the water content

is 24%. It is on average about 13% but only about 2% at the periphery. Presumably, these particles would still be slightly sticky because (a) stickiness (= the tendency to stick to the machinery) considerably increases with temperature; and (b) upon removal from the drying air the outside of the powder particles rapidly increases in water content due to internal exchange of water. Moreover, larger particles will be ''wetter,'' hence more sticky. But a powder with an average water content of about 8% can readily be discharged by means of cyclones.

Additional drying of the powder then is done in a fluid bed drier. Satisfactory fluidization of the powder can only occur if the drier is vibrated. In a spray drier the air inlet temperature is high; the holdup time of the powder is short, say, a few seconds. In a fluid bed drier the air inlet temperature is relatively low (e.g., 130°C), little air is consumed, and the residence time of the powder is much longer, i.e., several minutes. Because of this, a fluid bed drier is much more suitable for the final stages of drying. For example, a comparison between traditional and two-stage drying, using the same spray drier, the same skim milk concentrate with 48% dry matter, dried to the same water content of 3.5%, may yield the following:

	1	2
Number of stages	1	2
Inlet air temperature (°C)	200	250
Outlet air temperature chamber (°C)	94	87
a_w outlet air chamber	0.09	0.17
Total heat consumption (kJ/kg water)	4330	3610
Capacity (kg powder/h)	1300	2040

The efficiency of the heat expenditure thus is better (by 20%) and the capacity greater (by 57%); against this is the capital outlay for the fluid bed drier. The additional drying consumes only 5% of the heat. The quality of the powder (insolubility index) is certainly not poorer, but generally better.

A fluid bed offers additional opportunities. For instance, it is quite simple to add a cooling section. The bed can also be used for agglomerating purposes. The main incentive for agglomeration is that a fine powder poorly disperses in cold water (Section 17.5). Therefore, often an attempt is made to produce a coarse-grained powder. In the fluid bed the powder particles collide intensely with each other. As a result, they agglomerate if they are sufficiently sticky, i.e., have a high enough water content at their periphery. Hence, agglomeration is enhanced by blowing steam into the powder (this is called *rewetting*, which is mostly applied when producing skim milk powder). The air velocity in the fluid bed may be adjusted in such a way that the smallest powder particles (which have already become very dry and therefore show poor agglomeration) escape separation. The latter particles are fed back to the drying chamber, gain entrance to the atomized liquid, and become agglomerated with the drying droplets (especially applied for whole milk powder). This means of manufacture causes no

problems for the powder quality because the smallest particles show little heat damage. All of these procedures result in a powder that disperses readily, i.e., ''instant'' powder; to be sure, manufacture of instant whole-milk powder is more difficult (see Section 17.5).

Two-stage drying can also be done in the drying chamber. The conditions then must be made such that a fairly concentrated, more or less fluidized mass of powder is present at the bottom of the chamber and remains there for a longer time, while additional drying air is blown through that mass. Another type of equipment is a drier with an integrated conveying belt (filter mat drier). It has a moving perforated belt at the bottom of the drying chamber. Drying air is first blown downward through the powder deposited on the belt, and after that cooling air. This machinery allows removal of a larger part of the water by additional drying. After all, there is less risk of the incompletely dried powder sticking to the wall of the machinery, especially when drying whey. Filter mat driers are also suitable for high-fat products, which tend to be very sticky at high temperature.

9.4 MEMBRANE PROCESSES

In the application of a membrane process, a solution is enclosed in a system confined by a semipermeable membrane. Some components of the solution can pass the membrane; some cannot. The driving force may be a pressure difference over the membrane or a difference in electrical potential. The latter method refers to electrodialysis. In dialysis the driving force is a concentration difference or, more precisely, an activity difference. In microfiltration and ultrafiltration a relatively small pressure difference of, say, 1 bar is involved; in reverse osmosis a far higher pressure difference is applied. The liquid passing the membrane is called *permeate*; the retained solution concentrate or, rather, *retentate*.

Microfiltration is intermediate between common filtration and ultrafiltration. The pores in the membrane are fairly wide, i.e., >0.1 μm, and the pressure difference is small (see Fig. 9.24). The method may be used to remove small particles and microorganisms from cheese brine or wastewater, and is in principle also suitable to remove bacteria from skim milk (see Section 14.1.3). Microfiltration may especially be used if the amount of ''retentate'' is relatively small.

Ultrafiltration effectively separates macromolecules (e.g., proteins) and particles (casein micelles, fat globules, cells, bacteria, etc.) from the solution. The common aim is to accumulate protein. The process is often applied to whey and to skim milk. In principle, it can cause appreciable change in the composition of milk products, and it thus allows preparation of unconventional products. Alternatively, the milk may be concentrated to an extent as to approach the composition of curd (or quarg) and be clotted subsequently (see Section 22.2). Ultrafiltration is an almost unique process, though gel filtration can provide comparable
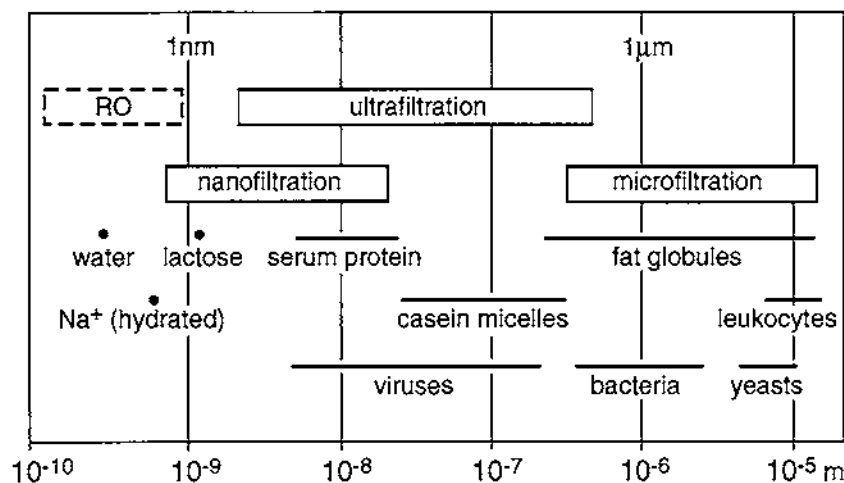
FIGURE 9.24 Approximate particle sizes for which separation by means of micro-, ultra-, and nanofiltration can be applied. Fundamentally, reverse osmosis (RO) does not separate on a particle size basis. The size of some molecules and particles in milk is also indicated.

results. However, industrial application of gel filtration incurs many problems and is costly.

Nanofiltration (see also Fig. 9.24) is used on an industrial scale to separate mixtures of proteins and peptides on a molecular size basis. Moreover, some nanofiltration membranes can be used for desalting, when high pressures are applied; this is an alternative to electrodialysis.

Reverse osmosis is applied to remove water, and it thus is an alternative to evaporation because it consumes far less energy (see Table 9.2). Its capital expenditure and maintenance costs are usually higher; hence, its profitability depends on conditions. The process is applied to whey, skim milk, and highly polluted wastewater, and has the advantage of operating at low temperature and of retaining a great deal of volatile substances. Disadvantages may be that milk cannot be highly concentrated and that the permeate is by no means pure water.

Electrodialysis is aimed at removing ions, e.g., in the preparation of dietary products, or as a step during the manufacture of a purified protein concentrate. The process is applied to partially demineralize whey. Passing milk over ion exchange columns with suitable resins also removes ions, but that method has several drawbacks, e.g., because the resin must be regenerated frequently.

9.4.1 Ultrafiltration

9.4.1.1 Composition of the Retentate

An ultrafiltration membrane is a filter with very narrow pores (mostly a good 1 nm in width) through which most molecules and ions can pass, whereas macromolecules and particles are retained. In principle, water activity, ionic strength, and pH are equal on either side of the membrane. In the retentate, protein accumulates and its properties, including conformation, remain essentially unaltered. The ratio between, for instance, protein and sugar in the retentate changes considerably. Consequently, a retentate of skim milk has a composition completely different from that of evaporated skim milk and, as a result, has different properties: it exhibits far weaker Maillard reactions during heating, is much more heat-stable at an identical protein content, and has a higher viscosity at identical dry matter content (Fig. 3.20B).

   The separation during ultrafiltration is less perfect than is suggested in the previous paragraph. Solvent, in this case water, passes through the membrane at a rate or flux q_w (kg · m^{-2} · s^{-1}), and solute, such as a component x, passes at a flux q_x. The concentration of x at the pressure side of the membrane is c^*. c^* can conveniently be expressed in kg per kg of water; c_w, the concentration of water, then equals 1. We define the reflection R^* of solute x as:

$$R^* = \frac{q_w - (q_x/c^*)}{q_w} = 1 - \frac{q_x}{q_w c^*} \tag{9.6}$$

Ideally, $R^* = 0$ for small molecules and $R^* = 1$ for protein. R^* is a function of molecular size, and that function depends on the type of membrane involved (see Fig. 9.25). R^* changes gradually with molar mass, partly because of a spread in pore width in a membrane, and differences in spread explain the differences in the slope of the curves. Even for identical pore widths, however, R^* will gradually change with molar mass. This is because the pores in the membrane exert a mechanical sieve action on the movement of even small molecules. The nearer the molecular size is to the pore width in the membrane, the greater the resistance. This is illustrated in Figure 9.26. If the pores cause the same resistance to the solute as to the water molecules, $R^* = 0$.

   R^* not only depends on the type of membrane, but for small molecules it also increases to some extent with the pressure difference Δp over the membrane; see Equation (9.9) below. Often, R^* also depends on the presence and thickness of a gel layer (see Section 9.4.1.2). A further complication is that activities in the solution rather than concentrations are the relevant variables in Equation (9.6). At high concentrations the difference may be considerable, partly because a relatively large proportion of the water is not available as a solvent.
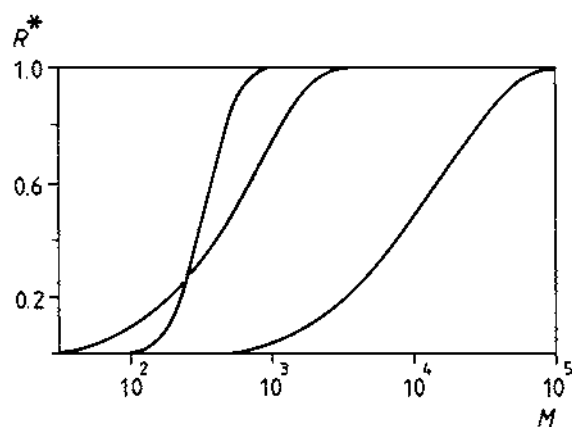
FIGURE 9.25 Dependence of the reflection R^* on molar mass (M). Approximate examples for three different membranes. After P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).
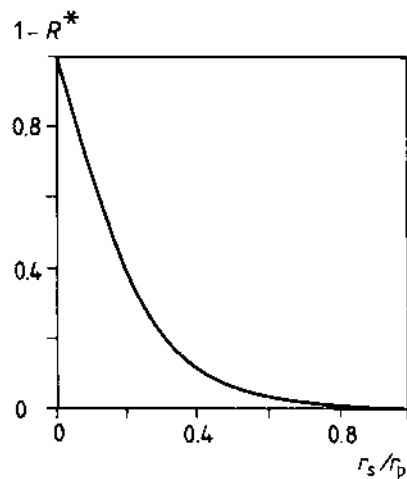
FIGURE 9.26 The relative passage velocity ($1 - R^*$) of a sphere with radius r_s through a cylindrical pore of radius r_p as a function of their ratio. Approximate calculated result.

In the ultrafiltration of skim milk and whey, low molar mass proteins or peptides are not fully retained ($R^* < 1$). Usually, for lactose $R^* = 0.02$–0.15, for citrate $R^* = 0.01$–0.10, and for smaller ions R^* is negligible. Anions in milk serum are on average larger than cations. The ensuing difference in flux has to be counterbalanced by a flux of hydroxyl ions, which causes the pH of the permeate to be 0.04–0.10 higher than that of the original liquid. Figure 9.27 illustrates some of the aspects mentioned. Calculated curves are drawn for $R^* = 1$ and $R^* = 0$. The curve for $R^* = 0$ is not horizontal. This is because at a constant and equal ratio of solute to water [c^* as defined in Equation (9.6)] on either side of the membrane, the solute concentration in the total volume (c in Fig. 9.27) decreases during concentration, since the water content of the retentate decreases. (In calculating the curve for $R^* = 0$, water not available as a solvent was not taken into account. Table 9.1 shows that nonsolvent water cannot be neglected. After making adjustments for water nonsolvent for lactose, point $R^* = 0$ for $Q = 40$ in Figure 9.27 would still be lower by about 0.1, if R^* for lactose is assumed to be zero.)

Figure 9.27 shows that there is no total reflection for protein. The reflection
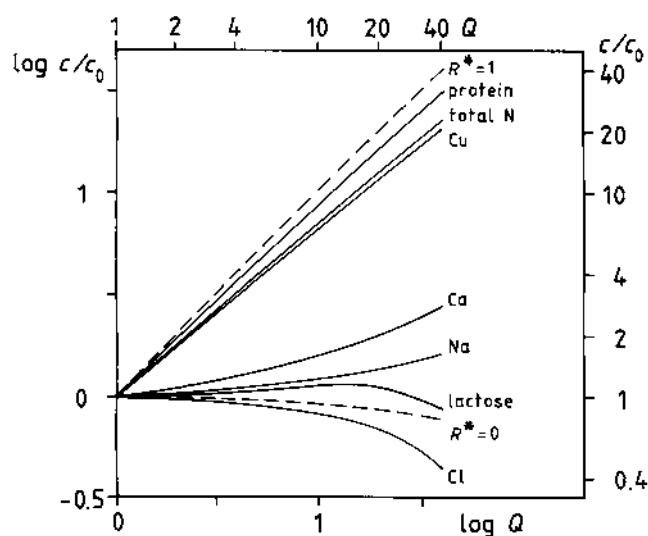
FIGURE 9.27 The ratio of the concentration (c, expressed per m³ of retentate) of some components in the retentate of ultrafiltered sweet whey to their original concentration (c_o) as a function of the concentration factor Q (=original volume/retentate volume). Approximate examples, mostly after J. Hiddink, R. de Boer and D.J. Romijn, *Neth. Milk Dairy J.* **32** (1978) 80.

for total N is still smaller because it comprises nonprotein nitrogen (NPN), for which $R*$ mostly approximates zero. (NPN constitutes about 25% of the nitrogen in whey!) Obviously, any component that closely associates with protein, such as Cu, is retained in the retentate, though to a lesser extent the same holds true for the counterions of the negatively charged protein, in this case cations. Figure 9.27 also shows that Ca^{2+} is present in a relatively higher concentration as a counterion in the diffuse double layer than Na^+, which is fully in line with theory. Mutatis mutandis co-ions, especially Cl^-, are more than proportionally removed with the permeate, resulting in $R* < 0$. This is because they have a decreased concentration in the diffuse double layer around the protein molecules.

So far we have discussed reflection. In actual practice, there is greater interest in retention R, as given by

$$R = 1 - \frac{c_p}{c_f} \tag{9.7}$$

where c_p and c_f are concentrations (e.g., in $kg \cdot m^{-3}$) in permeate and feed liquid, respectively. Initially, when the concentration factor Q (=original volume/retentate volume) scarcely surpasses 1, $R = R*$, but that does not remain so because at increasing Q the composition of the permeate changes. This reveals another difference between reflection and retention. $R*$ gives a state (flux ratios) at one moment, whereas R refers to the whole process and can be calculated by integration over the process time.

The retention changes even stronger by applying *diafiltration*. In diafiltration, water is added to the retentate after a certain Q has been reached, and ultrafiltration is continued. In this way, the ratio between protein and solutes in the retentate can be increased. Of course, this holds to a far lesser extent for the ratio of protein to counterions, and to components bound to protein. If a protein concentrate of low 'ash content' is to be made by using ultrafiltration, it is advisable to lower the pH appreciably before ultrafiltration. Table 9.4 gives examples of the composition of retentates obtained from whey. The composition of the permeate also depends on other conditions. The main variable in ultrafiltration usually is the membrane because that primarily determines the reflection for various constituents.

9.4.1.2   Permeate Flux

The flux is the quantity q of liquid that passes the membrane per unit time and surface area. Applying the equation of Darcy to a membrane yields

$$q = \left(\frac{B}{h}\right)\frac{\Delta p}{\eta} \tag{9.8}$$

TABLE 9.4 Composition of the Dry Matter of Retentate Obtained by Ultrafiltration of Whey[a]

Q	1	5	10	20	35	35[b]	20
Dry matter (%)	6.6	10	14	20	25	22	17
pH during ultrafiltration		6.6	6.6	6.6	6.6	6.6	3.2
Protein/dry matter (%)	12	34	45	58	70	82	59
Lactose/dry matter (%)	74	51	39	27	17	7	27
''Ash''/dry matter (%)	8	6	5	4	3.5	2.5	2.7
Citrate/dry matter (%)	2.5	1.8	1.7	1.6	1.4		
Fat/dry matter (%)	1	2	3	4	5	6	4

[a] Approximate examples. Q = concentration factor = volume reduction factor.
[b] Followed by diafiltration, i.e., water is added to increase the volume by a factor of 3, and the mixture is ultrafiltered again.

where B is permeability coefficient of the membrane, h is effective thickness of the membrane, Δp is pressure difference over the membrane, and η is viscosity of the permeating liquid. B is approximately proportional to the square of the pore width and to the surface fraction occupied by pores. Consequently, q increases by enlarging the pore width, but that is at the expense of the selectivity of the membrane. The flux mostly is not precisely proportional to Δp because at higher pressures B decreases due to compression of the membrane. Often, (B/h) is of the order of 10^{-12} m, which implies that the flux of water through an ultrafiltration membrane approximates $400 \text{ kg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ at $\Delta p = 100$ kPa (1 bar).

The flux achieved during ultrafiltration of whey or skim milk is many times lower than that during ultrafiltration of water. The following are possible causes.

a. The viscosity of the permeating liquid is higher than that of water, e.g., by 20%.
b. Protein molecules immediately adsorb onto the membrane, also in the pores, and thereby reduce the effective pore width. The effect is hard to determine precisely, but presumably it is considerable. Obviously, the narrowing of the pores increases the selectivity.
c. Part of the solutes is retained. Any retention of solute causes a difference in osmotic pressure $\Delta \Pi$ on either side of the membrane, resulting in a somewhat lower effective pressure difference $\Delta p - \Delta \Pi$. The decrease is small, say, 10%.
d. A concentration gradient is formed because liquid passes the membrane and part of the material in that liquid cannot pass. The gradient is counteracted by the mixing effect of the liquid moving along the membrane, but a certain gradient or a liquid layer with increased dry

matter content will be formed anyway. This is illustrated in Figure
9.28. The phenomenon is often (incorrectly) called concentration polar-
ization. It intensifies the effect mentioned in point c which, however,
remains small.

e.   The concentration gradient can, however, affect the permeate flux
     much more strongly if it increases near the membrane to such an extent
     that the concentration of some constituents, especially proteins, ex-
     ceeds their solubility. A layer of precipitated material then is formed
     on the membrane. This ''gel layer'' causes a further reduction of the
     flux, the more so the thicker the layer and the higher the pressure,
     because a higher pressure compresses the gel layer thereby narrowing
     its pores. Usually, a gel layer also improves the reflection of many
     constituents; in other words, it enhances the membrane selectivity.

The above-mentioned facts may explain the effects of some process and product
variables on the ultrafiltration flux, at least qualitatively. The composition of the
liquid at the pressurized side is paramount because that determines whether a gel
layer can be formed or not. The pH strongly affects the solubility of the protein.
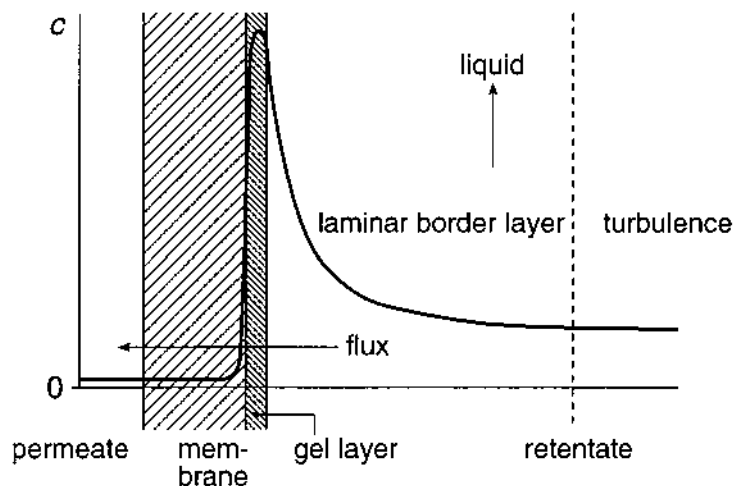
FIGURE 9.28 Concentration gradient and gel layer on a membrane along
which the concentrating liquid flows. Schematic. c = concentration of a sol-
ute. Usually only a thin layer of the membrane on the retentate side is effec-
tive as a filter. In ultrafiltration, the average liquid flow rate along the mem-
brane is generally about 10^6 times the flow rate across the membrane (the
flux).

Accordingly, the flux will be at a minimum near the isoelectric pH of the protein (Fig. 9.29). Moreover, the solubility of calcium phosphates is essential, because phosphates mostly are important constituents of the gel layer. Because of this, the following steps increase the flux: removal of calcium (e.g., by electrodialysis), increasing the pH to 7.5 (some calcium salts precipitate before the ultrafiltration and hardly enter the gel layer), and preheating (e.g., 30 min to 55°C, which has a comparable effect). However, the extent of concentration, expressed through, for instance, the protein content of the retentate, has an overriding effect on the flux. Once that concentration is high, a substantial gel layer always forms. This strongly slows down ultrafiltration, despite any measures taken. Incidentally, even a small release of permeate from a highly concentrated retentate causes appreciable further concentration (cf. Fig. 22.6).

Formation and thickness of the gel layer depend on the hydrodynamic conditions. This is because a concentration gradient can only form in the ''laminar border layer'' (Fig. 9.28). If there is no liquid flow along the membrane, a thick gel layer is formed at once and the flux is sharply reduced. If so, essentially ultrafiltration cannot be achieved. The intensity of the turbulence determines the thickness of the laminar border layer. The flow rate of the retentate thus has a considerable effect, and that holds also for the geometry of the space containing the retentate. The increase in viscosity due to concentration eventually also causes reduced turbulence; hence, a greater tendency to form a gel layer. Even at constant protein content, i.e., constant Q, the permeate flux eventually decreases, partly due to increasing gel layer density.

Increasing the flux through the membrane enlarges, ceteris paribus, the con-
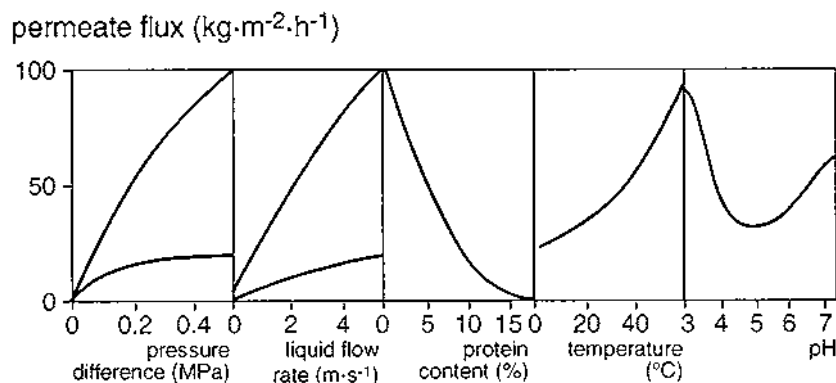
FIGURE 9.29 The influence of some process and product variables on ultrafiltration rate. Approximate examples for whey. Two curves drawn refer to different protein contents, where the upper one refers to nonconcentrated whey.

centration gradient. Microfiltration permits a far greater flux than ultrafiltration. Consequently, it requires a more intense turbulence, which means a faster circulating liquid, to prevent the rapid formation of a thick gel layer.

The influence of pressure difference and temperature on the flux (Fig. 9.29) is self-evident.

9.4.1.3 Technical Operation

In batchwise ultrafiltration, a certain quantity of liquid is put into the equipment and concentrated to the desired dry matter content. It is a drawback that the retentate decreases in volume whereby circulation, i.e., a rapid liquid flow along the membrane, becomes hampered. A simple flow-through system, as depicted in Figure 9.30A, prevents such problems from occurring. This system is especially suitable if concentration is moderate. If concentration is higher, and if diafiltration is involved, multiple-effect circulation is often used (Fig. 9.30B). Overpressures ranging from 0.1 to 0.5 MPa are usually used. To be sure, a higher concentration causes a larger part of the overpressure to be lost due to friction in the retentate; in other words, increasing energy is required to rapidly circulate the liquid. The
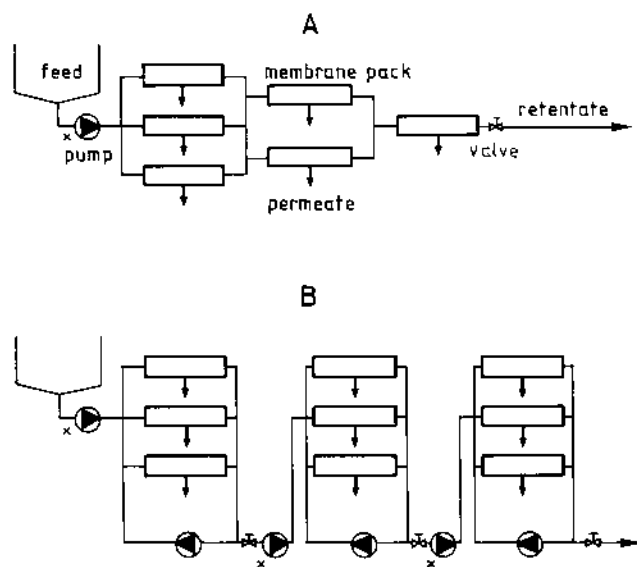
FIGURE 9.30 Arrangements for ultrafiltration or reverse osmosis. Schematic. (A) Simple flow. (B) Flow in several stages with recirculation. Pumps marked with x can resist considerable back pressure; the other pumps are for circulation.

viscosity of the retentate is therefore often limiting (also because a high viscosity diminishes turbulence, hence readily leads to a gel layer that further increases the frictional resistance). That is why skim milk rather than milk is ultrafiltered.

The structure supporting the membrane and the shape of the retentate space can vary widely. There are four main module types, the advantages and disadvantages of which are summarized in Table 9.5. The type of membrane involved (its structure and composition) is paramount. Cellulose acetate membranes cannot resist temperatures over 30°C and, accordingly, their actual usage is restricted to a low temperature (e.g., 10°C) because bacteria will grow excessively at 20–30°C. Moreover, these membranes cannot withstand low or high pH values, so they can only be cleaned by using enzyme preparations. Membranes from polyamide (nylon) and from several other polymers can withstand far higher temperatures and acidities. They allow ultrafiltration at such a high temperature (e.g., 55°C) that there is hardly any bacterial growth. The membranes can be cleaned by means of acid and alkali. The new ceramic membranes offer still better possibilities.

Cleaning and disinfection are paramount, and the equipment should be suitable for these treatments. Ultrafiltration takes a long time and bacterial growth

TABLE 9.5   Various Types of Ultrafiltration Units

Flat membrane (on supporting plates)
   Relatively small volume per m^2 membrane
   Great pressure loss due to flow resistance
   Can hardly be inspected without dismantling
   Dismantling (e.g., to replace a membrane) easily causes leaky membranes
Tubular membrane (in hollow supporting tubes)
   Large volume per m^2 membrane
   Low flow resistance
   Can easily be cleaned, inspected, and replaced
   Suited for ceramic membranes
Hollow fiber (no support)
   Small volume per m^2 membrane
   Only low pressures are possible (170 kPa overpressure)
   Fibers can foul
   Leakage requires replacing of a complete cartridge
Rolled flat membrane (pile of membranes alternated with flexible supports wound
     around a tube)
   Relatively small volume per m^2 membrane
   Low flow resistance
   Hard to inspect
   Leakage requires replacing a complete cartridge

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can easily occur in the retentate. The permeate is sterile unless one of the many membranes is leaky.

9.4.2 Reverse Osmosis

Reverse osmosis differs from ultrafiltration in the application of much higher pressures (3–10 MPa). It removes water against an osmotic pressure. The osmotic pressure Π is considerable. For nonconcentrated milk or whey, $\Pi \approx 0.7$ MPa, and it increases during concentration by removal of water according to $\Pi \approx 0.7\, Q^*$, where $Q^* =$ relative increase of the dry matter content in proportion to water (Section 9.1). Clearly, the membrane is semipermeable. It does not act as a filter with narrow pores but rather as a layer of material in which water can dissolve and through which it can pass, while most of the other (mainly hydrophobic) components cannot do so or can barely do so. Transport of a component occurs by diffusion through the membrane or, essentially, through the thin layer of the membrane, which is semipermeable, and the porous remainder of the membrane is merely a support. Consequently, Equation (9.8) does not apply. The rate of transportation is proportional to the solubility of the component in the membrane and to its effective diffusivity. The diffusion coefficient in the membrane decreases considerably with increasing molar mass of the diffusing component. Obviously, the retentate obtained by reverse osmosis differs somewhat from the concentrate after evaporation, and the permeate is by no means pure water. The reflection coefficient R^* [Equation (9.6)] of small molecules is $0.75 - 0.99$, greatly varying with the composition of the membrane. Urea can pass to some extent, and even lactose and low molar mass peptides may do so. Accordingly, bacteria can grow in the permeate. Volatile flavor substances are satisfactorily retained.

R^* of most components increases with the pressure difference Δp applied over the membrane and decreases with increasing difference in osmotic pressure $\Delta \Pi$ on either side of the membrane (hence, with increasing retentate concentration). For an aqueous solution of solute x, the water flux will be proportional to $\Delta p - \Delta \Pi$ and the flux of x to $\Delta p + \Delta \Pi$. In other words, Equation (9.6) should be adjusted as follows:

$$R^* = 1 - \frac{(q_{x,0}/c^*)\,(1 + \Delta\Pi/\Delta p)}{q_{w,0}\,(1 - \Delta\Pi/\Delta p)} \tag{9.9}$$

where $q_{x,0}$ and $q_{w,0}$ are the solute and water fluxes, respectively, for the hypothetical case that $\Delta\Pi = 0$. For example, $\Delta\Pi/\Delta p$ initially is about 0.2, and it becomes 0.8 toward the end of the process. Suppose now that $R^* = 0.9$ for $\Delta\Pi = 0$. Then, according to Equation (9.9), R^* decreases during the process from 0.85 to 0.1. R^* of some components can even become negative if the liquid is highly concentrated. Fortunately, R^* of most solutes is much higher, and it decreases,

for instance, from 0.985 to 0.91 when $\Delta\Pi/\Delta p$ changes from 0.2 to 0.8. Moreover, the total amount of permeate released toward the end of the process is only small. On the other hand, a concentration gradient developing near the membrane (Fig. 9.28) raises the effective $\Delta\Pi$ above the average value. Furthermore, advanced concentration reduces the retention R at constant reflection R^*. All in all, the last bit of permeate released during reverse osmosis may be more like ultrafiltration permeate than like water. This, of course, depends on the type of membrane involved. (The above influence of Δp and retentate concentration on R^* applies also to ultrafiltration, though to a far lesser extent because the actual $\Delta\Pi$ is smaller and initially even zero.)

Applying reverse osmosis to whey or skim milk results in a lower flux than when pure water is used. This is comparable to conditions during ultrafiltration; however, among the causes listed in Section 9.4.1 the first two will hardly have an effect because the viscosity of the permeate is and remains similar to that of water and there are no pores that can foul. The increase of the osmotic pressure, on the other hand, is of paramount importance. The water flux is proportional to $\Delta p - \Delta\Pi$ (see above). Suppose that skim milk is concentrated to 30% dry matter (a higher concentration is usually not reached) and that a pressure difference Δp = 4 MPa is applied. Initially, $\Delta p - \Delta\Pi = 4 - 0.7 = 3.3$ MPa. For 30% dry matter, $Q = 30/9.3 = 3.23$ and $Q^* = 4.18$. This will lead to $\Delta\Pi \approx 2.9$ MPa, hence $\Delta p - \Delta\Pi = 1.1$ MPa. In other words, the effective pressure difference is reduced to one-third or even less as the concentration gradient near the membrane can increase considerably. Finally, a gel layer may form, especially at high Δp, which implies a large flux, hence a strong concentration gradient. This layer reduces the flux still further. Figure 9.31A illustrates all of these aspects.

Figure 9.31B gives examples of the flux at increasing concentration, as obtained during reverse osmosis of some liquids. The differences involved greatly depend on differences in formation of a gel layer. Micellar casein readily forms such a layer. The proteins in whey do not form a gel layer at pH ≥ 6, but they do at pH = 4.6, at which point they are far less soluble. Calcium phosphate is saturated in milk and whey; accordingly, it plays a part in the formation of a gel layer, especially in whey at neutral pH. This is because casein can accommodate insoluble calcium phosphate far better than serum proteins do. At pH = 6, calcium phosphate is less readily supersaturated. Therefore, in reverse osmosis of whey the following results for the water flux (in kg $\cdot$ m$^{-2} \cdot$ h^{-1}) may be found:

pH of whey	6.6	6.0	4.6
Initial flux	37	37	27
Flux when $Q \approx 3$	5	20	15

Figure 9.31B also shows that the flux remains high during reverse osmosis of an ultrafiltrate of skim milk or whey. This is because little, if any, gel layer is formed. Accordingly, a much higher Δp can be applied without the flux decreas-

flux (kg·m⁻²·h⁻¹)
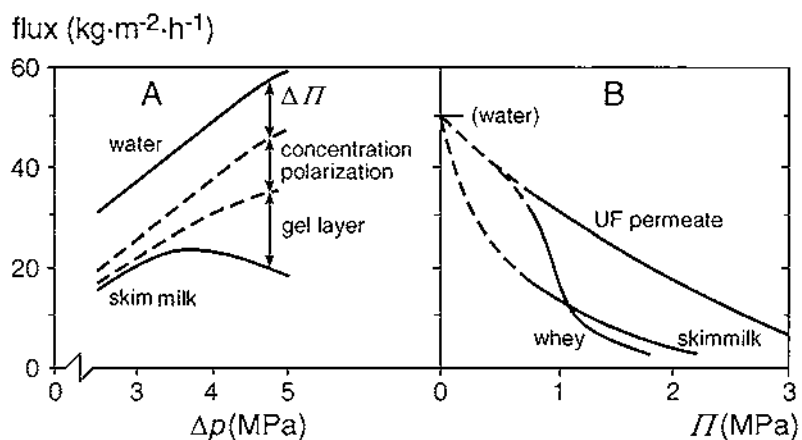
FIGURE 9.31   The effect of some process and product variables on the permeate flux during reverse osmosis. Approximate examples for pH $\approx$ 6.7 and 30°C. (A) Flux of pure water and slightly concentrated skim milk ($Q^* \approx$ 1.5) as a function of the pressure difference Δp. The probable causes of the flux difference are shown schematically. (B) Flux of some liquids as a function of the concentration, expressed in the osmotic pressure Π of the retentate. From J. Hiddink, R. de Boer and P.F.C. Nooy (slightly modified), *J. Dairy Sci.* **63** (1980) 204.
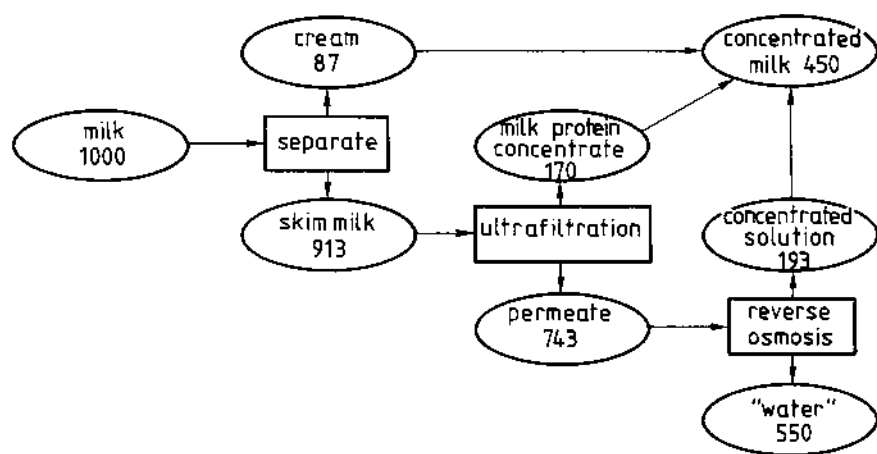
FIGURE 9.32   Principle of the concentration of milk via centrifugal separation, ultrafiltration, and reverse osmosis. The numbers are examples of the quantities in kg.

ing again (as in Figure 9.31A). A way of processing as depicted in Figure 9.32 is therefore often used. This processing requires additional equipment but yields a higher flux. Concentration to $Q > 2.2$ is, however, almost impossible.

Reverse osmosis is usually not applied to milk. First, the fat globules increase the viscosity and, second, the globules are easily homogenized when the retentate flows out of the equipment through the pressure release valve. Such homogenization causes considerable lipolysis if raw milk is involved. Gradual release of pressure via discharge of the concentrate through a long capillary tube can prevent the homogenizing effect. Furthermore, high concentration of the milk causes crystallization of lactose and subsequent blockage of the equipment, at least at low temperature. If the membrane does not endure high temperatures, a concentration greater than about 22 g lactose/100 g water (Fig. 2.3, i.e., $Q^* \approx$ 4.2) cannot be achieved. If applied to whey, a dry matter content of about 24% can be reached.

Reverse osmosis is technically carried out in much the same way as ultra-filtration. The higher pressures applied necessitate several adaptations, e.g., to the pumps and to the membrane support. The flux increases with the temperature, almost as strongly as the viscosity of water decreases with temperature (Table 3.4).

9.4.3 Electrodialysis

Electrodialysis can be carried out in various ways, but membranes that let pass cations only or anions only are generally used. Figure 9.33 represents the principle. It thus is an open system; a salt solution flows along the electrodes and also serves to remove the formed gas. The number of parallel membranes is large, and their mutual distance is about 1 mm. During electrodialysis, concentration polarization occurs and a gel layer forms (e.g., from calcium phosphates and small peptides) but constant flushing of the equipment may maintain an acceptable capacity.

In demineralizing whey, the ash content is reduced by, say, 80%. Any further reduction requires excessive electrical energy. Different salts are removed at widely differing rates. Figure 9.34 gives examples. Obviously, the removal of a salt depends primarily on its ionization (Table 2.8) and therefore on the pH. That explains why at low pH lactate can hardly be removed. Furthermore, coions (e.g., Cl^-) will be more readily removed than cations (e.g., Ca^{2+} and Mg^{2+}), which preferably occur as counterions of the proteins. Moreover, the mobility of the various ions in a potential field varies. For instance, the mobility of H^+ is 32×10^{-8}, that of most monovalent ions in milk 6–7×10^{-8}, but that of Na^+ $4.4 \times 10^{-8} \ m^2 \cdot V^{-1} \cdot s^{-1}$. The greater the mobility, the better the removal. Finally, the permeability of the membrane for various ions can vary. Accordingly, the selectivity of electrodialysis markedly depends on conditions.
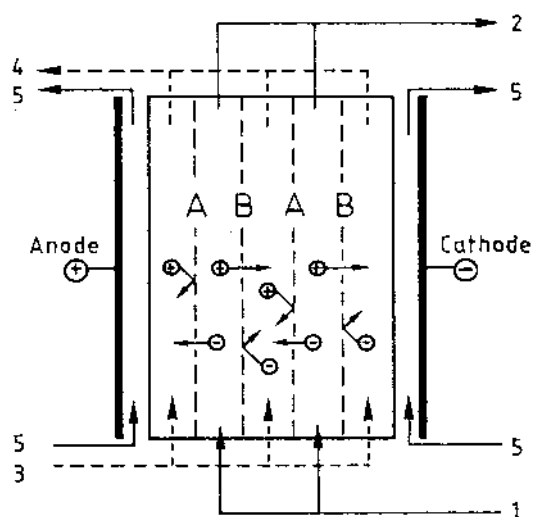
FIGURE 9.33 Principle of electrodialysis. Membrane A allows anions to pass, membrane B cations. 1, liquid to be demineralized; 2, demineralized liquid; 3, absorption liquid; 4, liquid with absorbed salts; 5, electrolyte solution.
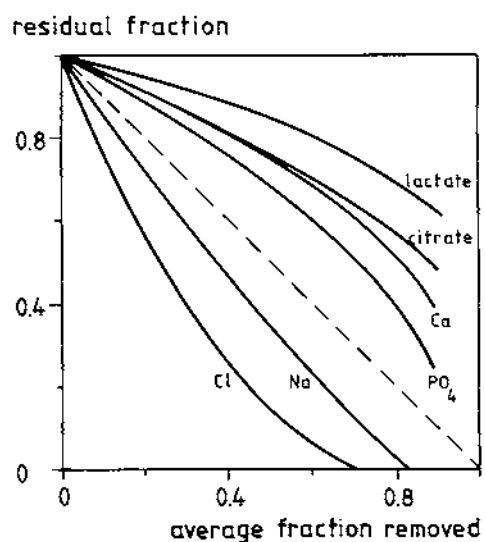
FIGURE 9.34 Example of demineralization of acid whey (pH ≈ 5.2) by electro-dialysis as a function of the quantity removed.

SUGGESTED LITERATURE

- General aspects of water activity and its effects on food stability are discussed by:

  O. R. Fennema, in: O. R. Fennema, ed., *Food Chemistry*, 3rd ed., Marcel Dekker, New York, 1996, Chapter 2.
- Concentrating and drying are discussed in several books on chemical and food process engineering such as:

  D. R. Heldman and D. B. Lund, eds., *Handbook of Food Engineering*, Marcel Dekker, Inc., New York, 1992.
- Principles of concentration and drying of foods are treated by:

  M. Karel, *Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975, especially Chapters 7–10.
- General aspects of spray drying are discussed by:

  K. Masters, *Spray Drying Handbook*, 5th ed., Longman, Harlow, 1991.
- Basic aspects of membrane processes are treated in:

  P. Mearen, ed., *Membrane Separation Processes*, Elsevier, Amsterdam, 1976, especially in Chapters 1–4.
- Membrane processes, including ultrafiltration and reverse osmosis, are also described by:

  F. A. Glover, P. J. Skudder, P. H. Stothart, and E. W. Evans, *J. Dairy Res. 45*, 1978, 291–318.

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  J. Hiddink, R. de Boer, and P. F. C. Nooy, *J. Dairy Sci. 63*, 1980, 204–214.

and in:

  New Applications of Membrane Processes, IDF Special Issue 9201, 1992.

10

Cooling and Freezing

10.1 COOLING

Often the purpose of cooling milk is to bring it to a temperature at which the rate of a process is influenced. For example, cooling is applied to retard spoilage, to induce crystallization of milk fat, or to enhance the creaming tendency of milk.

Much of what has been said about heating (in Chapter 6) also applies to cooling. In principle roughly the same equipment is used. Heat transfer may be slower because the higher viscosity of the liquid at the lower temperature causes the Reynolds number (Re) to be smaller (see Section 6.5). This especially causes problems in high-fat cream; in the streaming cream partial coalescence (clumping) of fat globules can occur as a result of high velocity gradients, and this tends to increase the viscosity of the cream even further. As a result, the coefficient of total heat transfer k_h (see Table 6.9) can fall below $100 \ W \cdot m^{-2} \cdot K^{-1}$. The clumping of the fat globules is also undesirable with respect to product properties. Moreover, the resistance to flow through a plate apparatus becomes excessive, and another type of heat exchanger is needed for high-fat cream. This means that cooling is relatively expensive, especially when chilled water (''ice water'') or chilled brine should be applied rather than well water with a temperature of, say, 11°C. A larger temperature difference between the brine and the incoming product usually does not greatly enhance the heat transfer rate because it results in local freezing of the product.

Cooling of packed products can also take a long time, especially if it involves viscous products. For the most part, air cooling is applied.

10.2   FREEZING

When milk is cooled it starts to freeze at about $-0.54°C$, if no supercooling occurs. Concentrated milk naturally has a lower freezing point. Pure ice is formed, and the remaining milk thus becomes concentrated, thereby further decreasing the freezing point. The lower the temperature, the higher the proportion of the water that freezes (Fig. 10.1). All of the consequences of concentrating mentioned in Section 9.1.3 apply. The water activity is a function of temperature only, if there is equilibrium among water, ice, and moist air, because the vapor pressure of ice, and hence its a_w, is a function of temperature only (for constant pressure). The relation is given in Figure 10.1.

     The change in composition of the remaining solution is considerable. At $-8°C$, for example, $Q^* \approx 13$, corresponding to about 60% dry matter when skim milk is frozen. Making an ultrafiltrate of the remaining liquid (at $-8°C$), it has a pH (at room temperature) of about 1 unit lower than that of an ultrafiltrate of the skim milk before freezing. The ionic strength may be almost 10-fold higher, the calcium ion activity is significantly increased, and the quantity of calcium phosphate in the casein micelles is also increased, despite the decrease in pH.

     Figure 10.1 pertains to equilibrium conditions, but if milk is quickly frozen, lactose (which becomes saturated near $-2°C$) will not crystallize at all or will hardly do so. Obviously, to maintain the same water activity less ice is formed,
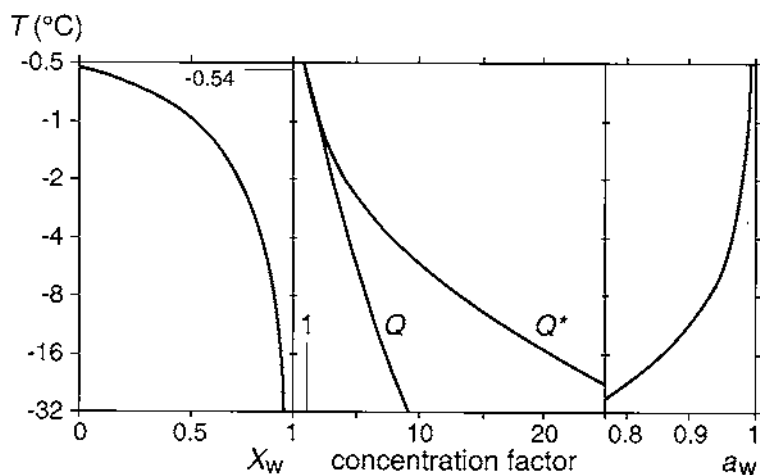
FIGURE 10.1   Freezing of skim milk. Fraction of the water frozen (x_w), concentration factor, and water activity (a_w) as a function of temperature (T). Approximate examples at equilibrium.

causing a less concentrated salt solution. When the temperature is below a certain value, presumably $-23°C$, the nonfrozen material is in the glassy state. This means that the diffusion coefficients are virtually zero. Consequently, the product remains nearly unaltered, partly because of the low temperature itself. Further crystallization of lactose does not occur. Autoxidation of lipids is still possible.

After frozen milk is thawed, aggregation of micellar casein may become manifest. This is partly because casein is salted out. Stirring for some time may redisperse the casein. Often, the coagulation is partly irreversible, presumably because micellar calcium phosphate has been irreversibly changed. If the milk is frozen quickly to below $-23°C$, lactose does not crystallize at all and irreversible coagulation of casein does not occur. A high concentration of sugar improves the stability of frozen milk because it decreases the ionic strength and the supersaturation with respect to calcium phosphate.

Freezing and subsequent thawing of milk causes part of the fat globules to clump. When cream is frozen, the effect will be much stronger. The cause of clumping is that ice crystals damage fat globules mechanically. Homogenization before freezing and rapid freezing diminish the loss of stability of the fat emulsion.

SUGGESTED LITERATURE

- Aspects of freezing are discussed in:
  O. R. Fennema, W. D. Powrie, and E. H. Marth, *Low-Temperature Preservation of Foods and Living Matter*, Marcel Dekker, New York, 1973. See especially Chapter 5, Part I.

11

Lactic Fermentations

Fermented milks are very old products. If raw milk is kept, it spoils by microbial action. At moderate temperatures, lactic acid bacteria generally are predominant, and the milk becomes ''spontaneously'' sour. When the sour milk is used and fresh milk is put in the same vessel without rigorous cleaning of that vessel, the fresh milk is ''inoculated'' with the remaining bacterial flora. The milk now sours more quickly, and generally due to a smaller number of bacterial species and strains. If this process is repeated under fairly constant conditions (especially temperature), natural selection leads to an almost pure lactic acid fermentation, although some other bacteria may remain present. The process can be improved by rigorously cleaning the vessel, heat-treating the milk to kill undesirable microbes, and inoculating the milk with a little bit of the sour milk from the previous batch; this then acts as a ''starter'' for the fermentation. The fermented milk thus obtained has a longer keeping quality and, often, a pleasant flavor. It is also much safer to the consumer because pathogenic bacteria have been killed and contamination with pathogens afterward can almost never lead to growth of these organisms. Moreover, individuals suffering from lactase deficiency (see Section 20.1) can tolerate the product quite well.

As a result of variations in conditions, a great number of fermented milk types have developed. Variables include species of milch animal, heat treatment of the milk, fermentation temperature, inoculum percentage, and concentrating of the milk. According to these conditions, various types of lactic acid bacteria become predominant, e.g., producing various flavor components. Most types contain two to four types of bacteria. In some products, yeasts or molds participate

in the fermentation. Often some kind of protocooperation between organisms occurs.

Nearly all types of fermented milks are the result of a very long evolution. Modern manufacture makes use of carefully selected and grown starters, and strictly hygienic processing is applied. Fermented milks are very popular products. In this chapter, some of the basic microbial aspects are discussed, as well as some process steps. Manufacture of fermented milks, with some emphasis on yogurt, is treated in Chapter 20. Lactic acid fermentation is also of importance for cultured butter (Chapter 19) and is essential in the manufacture and ripening of cheese (Part IV of this book).

11.1 LACTIC ACID BACTERIA: TYPES

Lactic acid bacteria are the prime agents in producing soured (fermented) milk and milk products. Table 11.1 lists the main types of bacteria applied. Table 11.2 shows important properties. All of the organisms applied in starters belong to the genera *Streptococcus, Lactococcus, Leuconostoc*, and *Lactobacillus*. These are gram-positive bacteria, which means that they are equipped with a thick cell wall.

Based on their morphology, lactic acid bacteria can be classified into cocci and rods. According to the optimum temperature for growth, a distinction is made between mesophilic organisms, which grow fastest at 20–30°C, and thermophilic ones, at 35–45°C.

11.1.1 Sugar Metabolism

Sugar metabolism involves transport of sugar into the cell and its further breakdown.

(1) *Uptake of sugar into the bacterial cell and formation of hexose mono-phosphates*. During growth in milk, lactose must be transported across the cell membrane. The following are possible mechanisms (see Fig. 11.1):

a. *A phosphoenol pyruvate–dependent phosphotransferase system (PEP/PTS)*. Lactose is transformed into lactose-P as it is transported into the cell. Inside the cell, phospho-β-galactosidase (P-β-gal) hydrolyzes the lactose-P to glucose and galactose-6-P. The glucose moiety is converted to glucose-6-P. Both sugar phosphates are metabolized further.

b. *An ATP-dependent permease system*. Lactose is transported as such and is hydrolyzed into glucose and galactose by β-galactosidase (β-gal or lactase). The glucose moiety is converted to glucose-6-P. Galactose-fermenting bacteria form glucose-6-P from galactose by the Leloir pathway.

TABLE 11.1 Approximate Classification[a] of Some Important Lactic Acid Bacteria Involved in Fermenting Milk and Milk Products

Family	Lactose fermentation	Genus	Species	10°C	45°C	35°C	40°C	50°C	10°C	37°C	15°C	45°C	Remarks
Streptococcaceae (cocci)	Homofermentative	*Streptococcus*	*S. thermophilus*	–	+								Thermophilic
		Lactococcus	*L. lactis* ssp. *lactis*	+	–								Mesophilic; lactococci
			L. lactis ssp. *lactis* biovar. *diacetylactis*	+	–								
			L. lactis ssp. *cremoris*	+	–								
		Pediococcus[b]	*P. pentosaceus*			+	+	–					Mesophilic
			P. acidilactici			+	+	+					Thermophilic
	Heterofermentative	*Leuconostoc*	*L. mesenteroides* ssp. *cremoris*						+	–			Mesophilic
			L. lactis						+	+			
Lactobacillaceae (rods)	Homofermentative	*Lactobacillus*	*L. helveticus*								–	+	Thermophilic
			L. delbrueckii ssp. *bulgaricus*								–	+	
			L. delbrueckii ssp. *lactis*								–	+	
			L. acidophilus								–	+	
			L. casei[b]								+	–	Mesophilic
			L. plantarum[b]								+	–	
	Heterofermentative	*Lactobacillus*	*L. kefir*[b]								+	–	Mesophilic
			L. brevis[b]								+	–	
			L. fermentum[b]								–	+	Thermophilic

[a] *Bergey's Manual of Systematic Bacteriology*, Vol. 2, 1986.
[b] Not intentionally used in starters but can be present as contaminants. *L. casei* and *L. plantarum* often play a role in cheese ripening, the other bacteria rarely.

TABLE 11.2 Important Properties of Lactic Acid Bacteria Mentioned in Table 11.1

| Property | Lactococcus lactis ssp. | | | Leuconostoc | | Streptococcus thermophilus | Lactobacillus | | | |
	lactis	lactis biovar. diacetylactis	cremoris	mesenteroides ssp. cremoris	lactis		delbrueckii ssp. bulgaricus	helveticus	delbrueckii ssp. lactis	acidophilus
Temperature for growth (°C)										
Minimum	8–10	8–10	8–10	4–10	4–10	20	22	20–22	18	20–22
Optimum	28–32	28	22	20–25	20–25	40	40–45	42	40	37
Maximum	40	40	37–39	~37	~37	50	52	54	50	45–48
Homofermentative	+	+	+			+	+	+	+	+
Heterofermentative				+	+					
Lactic acid production:										
% in milk (24 h at optimum temperature)	~0.9	~0.9	~0.9	tr	~0.8	~0.9	~2.5	~2.5	~1.2	~1
Dextro-rotatory, L(+)	+	+	+			+		+		+
Levo-rotatory, D(−)				+	+		+	+	+	+
Citrate										
Metabolized		+		+	+					
Formation of CO₂		+		+	+					
Formation of diacetyl		+		+	+	tr	tr			
Formation of acetaldehyde		+				tr	+			
Formation of exopolysaccharides	+/−		+/−			+	+			

| Key enzymes | GLYCOLYTIC PATHWAY | TAGATOSE PATHWAY | PHOSPHOKETOLASE PATHWAY | LELOIR PATHWAY | Key enzymes |
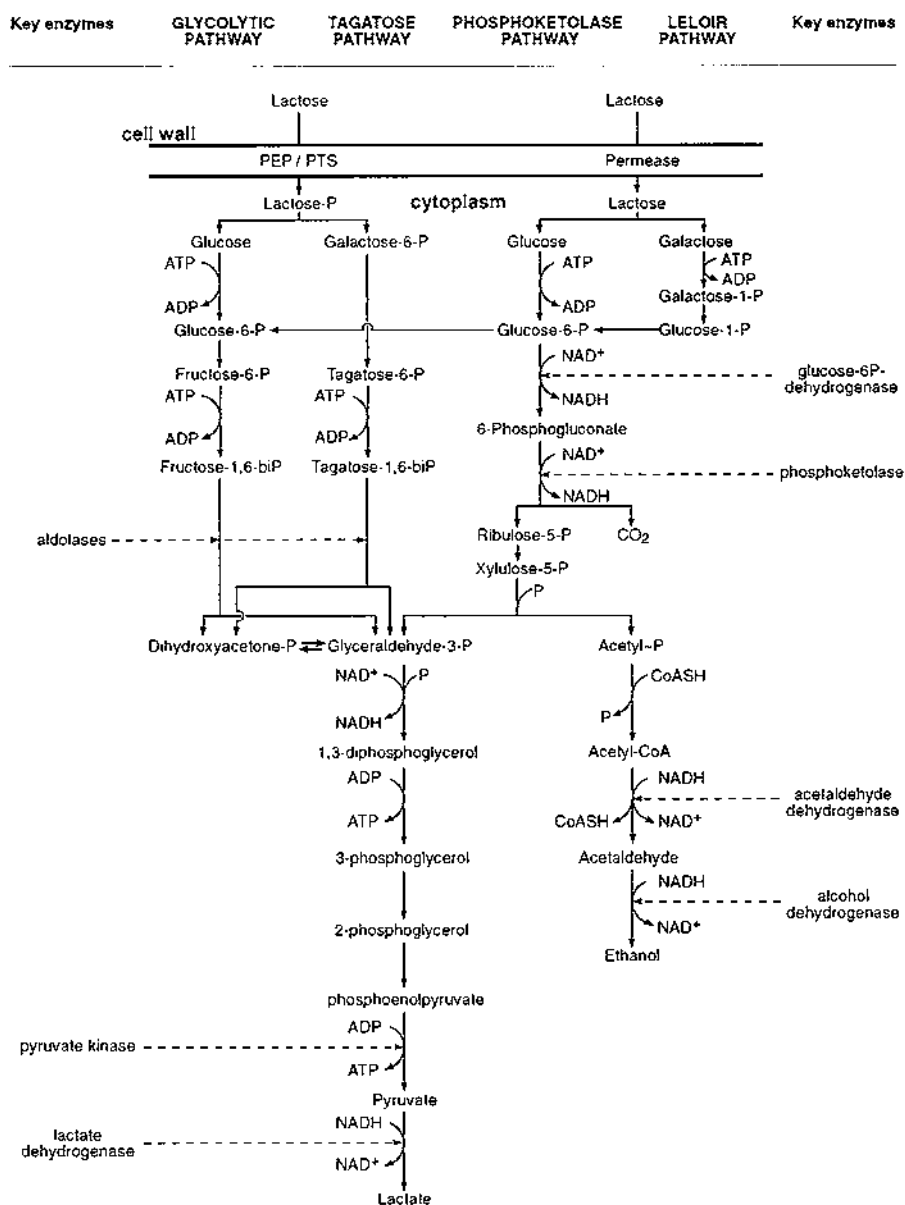
FIGURE 11.1   Metabolism of lactose in lactic acid bacteria. Modified after T.M. Cogan and C. Hill (In: P.F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology, Vol. 1, General Aspects, 2nd ed.*, 1993).

As a rule, lactic acid bacteria have both mechanisms of transport, but their relative importance varies widely. In most mesophilic homofermentative bacteria the PEP/PTS transport system predominates; especially in the lactococci β-gal activity is very weak. The permease system is dominant in the thermophilic homofermentative bacteria. In *Streptococcus thermophilus* there is no P-β-gal activity at all. If enzymes of the Leloir pathway are lacking, galactose is not hydrolyzed; this holds for many strains of *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Galactose then is secreted or is converted to (exo)polysaccharides. In most heterofermentative bacteria, the permease is the predominant transport system.

(2) *Further metabolism.* Homofermentative and heterofermentative lactic acid fermentations can be distinguished (see below). Pathways are shown in Figure 11.1. Every pathway involves consecutive reaction steps catalyzed by several enzymes. The free energy content of a metabolite is lower than that of the preceding product (a thermodynamic prerequisite), but not every reaction step provides useful energy. Nevertheless, the sequence of the reactions is such that most of the free energy transfer occurs in one step, leading to formation of an energy-rich phosphate, i.e., adenosine triphosphate (ATP), a coenzyme responsible for the transport of phosphate and energy. Formation of ATP from ADP (=adenosine diphosphate) occurs at the expense of a reduction of nicotinamide adenine dinucleotide (NAD^+), a coenzyme essential for transport of electrons and hydrogen, to NADH. NADH has to be oxidized again for the fermentation to proceed; this is because the amount of NAD^+ in the bacterial cell is very small.

All of the lactic acid bacteria show the same initial formation of hexose monophosphates from lactose. The further breakdown processes, however, vary with the species considered, and they result in various end products, discussed below.

11.1.1.1 Homofermentative Lactic Acid Fermentation

The sugar is metabolized via the glycolytic or Embden-Meyerhof pathway; galactose-6-P enters by the tagatose pathway. The following are key enzymes in the fermentation:

  a. *Aldolases*, necessary to hydrolyze hexose diphosphates to glyceralde-hyde-3-P.
  b. *Pyruvate kinase (PK)*, essential for the formation of pyruvate from PEP (PEP then loses its phosphorylating capacity in the PTS system).
  c. *Lactate dehydrogenase (LDH)*, essential for lactic acid production from pyruvate.

Regulation of the activities of all of these enzymes links the uptake of sugar to its metabolism. Important factors involved are:

a. Formation of PEP activates the uptake of sugar through the PEP/PTS system.
b. Intermediate phosphorylated metabolites up to glyceraldehyde-3-P, especially hexose diphosphates, activate PK and LDH.
c. NADH, formed during hydrolysis of glyceraldehyde-3-P, should be oxidized by lactic acid production from pyruvate

As a result of these factors, the essentially homofermentative lactic acid bacteria behave as ''lactic acid pumps'': if glycolysis is optimal, they convert 90% to 95% of the sugar to lactic acid. If glycolysis is minimal, especially if there is no sugar left, the amounts of hexose diphosphates decrease. Because of this, the PK and LDH activities decrease also. Moreover, accumulation of inorganic phosphate in the cell slows down any PK activity present. This results in a temporary piling up of PEP and of its precursors 3-phospho- and 2-phosphoglycerol (PEP potential). When sugar again becomes available, the inorganic phosphate content decreases and the PEP potential is immediately and fully used for the sugar uptake.

One disaccharide molecule yields two hexose molecules. Two molecules of glyceraldehyde-3-P are formed from one hexose molecule. Figure 11.1 shows that lactose is fermented according to

$$\text{Lactose} + 4H_3PO_4 + 4ADP \rightarrow 4 \text{ lactic acid} + 4ATP + 3H_2O$$
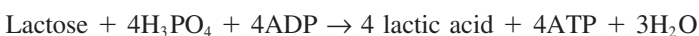
11.1.1.2 Heterofermentative Lactic Acid Fermentation

Heterofermentative lactic acid bacteria, including leuconostocs, lack aldolases and therefore cannot ferment sugar via the glycolytic pathway. The presence of glucose-6-P-dehydrogenase and of phosphoketolase permits metabolism by the 6-P-gluconate pathway. Phosphoketolase hydrolyzes 6-P-gluconate to CO_2 and a pentose-5-P, which, in turn, is converted to glyceraldehyde-3-P and acetyl-P. The conversion of glyceraldehyde-3-P to lactic acid is by the glycolytic pathway; acetyl-P should be hydrolyzed to ethanol if other hydrogen acceptors are lacking. Under these conditions, lactose is fermented according to

$$\text{Lactose} + 2H_3PO_4 + 2ADP \rightarrow$$
$$2 \text{ lactic acid} + 2 \text{ ethanol} + 2CO_2 + 2ATP + H_2O$$
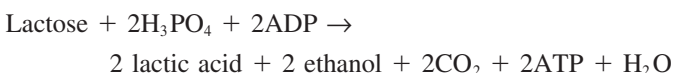
Formation of ethanol requires much energy. Accordingly, the presence of another hydrogen acceptor causes a preferential conversion of acetyl-P to acetic acid (see below).

11.1.1.3 Alternative Metabolic Pathways

Other end products besides those of the homofermentative and heterofermentative fermentations may be formed if NADH can be oxidized in another way, or if an additional source of pyruvate becomes available without supplying NADH. Important alternative pathways are (Fig. 11.2):

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PYRUVATE DEHYDROGENASE PATHWAY

c* pyruvate → CO₂ / TPP → hydroxyethyl-TPP → CoASH, NAD⁺, NADH → acetyl~CoA

1 a citrate → acetate
oxalacetate → CO₂ → pyruvate
lactose → pyruvate
pyruvate → NADH NAD⁺ → lactate
pyruvate → CO₂ / TPP (a)
[acetaldehyde-TPP]
CoASH / lipoic acid → TPP (c*)
acetyl~CoA
CoASH ···· (b) ···· formate
acetaldehyde → NADH NAD⁺ → CoASH
NADH NAD⁺ → ethanol
acetyl~CoA → P, CoASH → acetyl~P → ADP ATP (d) → acetate
pyruvate → TPP → acetolactate → CO₂
2
diacetyl → O₂ → CO₂
acetolactate → acetoin → 2,3-butyleneglycol
diacetyl → NADH NAD⁺ → acetoin 3
acetoin → NADH NAD⁺ → 2,3-butyleneglycol 4

(1) *Via the pyruvate-formate lyase (PFL) reaction.* In the homofermentative lactic acid bacteria, this enzyme is essential for the anaerobic pyruvate metabolism leading to formate and acetyl-CoA. The latter component is further metabolized into ethanol and acetate, in a ratio dependent on the redox potential. Formation of acetate yields ATP. In most of the lactococci, the PFL system is only activated when the sugar is nearly exhausted and the activities of the enzyme inhibitors hexose phosphate and triose phosphate decrease. In certain thermophilic streptococci the enzyme is in the active form even when there is an excessive amount of sugar present; ''strictly'' anaerobic conditions are required because PFL is rapidly inactivated if O_2 is present.

The PFL action accounts for the formate production by *Streptococcus thermophilus*, of importance in yogurt manufacture (Section 20.3).

(2) *Via the actions of NADH oxidase and NADH peroxidase, in the presence of O_2.* Most of the lactic acid bacteria applied in dairy products are microaerophilic, which means that the bacteria can (start to) grow at low oxygen pressure. During growth, the oxygen pressure decreases as a result of the action of NADH oxidases and NADH peroxidases according to the following:

$$NADH + H^+ + O_2 \xrightarrow{\text{NADH : H}_2\text{O}_2 \text{ oxidase}} NAD^+ + H_2O_2$$

$$2\,NADH + 2\,H^+ + O_2 \xrightarrow{\text{NADH : H}_2\text{O oxidase}} 2\,NAD^+ + 2\,H_2O$$

$$NADH + H^+ + H_2O_2 \xrightarrow{\text{NADH peroxidase}} NAD^+ + 2\,H_2O$$
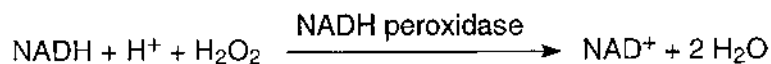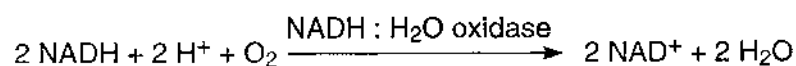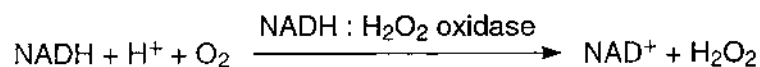
The overall consequences of these reactions are as follows:

    a.   The redox potential becomes much lower.

FIGURE 11.2 Pyruvate metabolism in lactic acid bacteria. The common metabolic pathways result in production of lactic acid or lactic acid plus ethanol, as is shown in Figure 11.1. Important alternative pathways are: a. Citrate metabolism. Enzymes involved include citrate lyase (1), acetolactate synthase (2), diacetyl reductase (3), and acetoin reductase (4). b. Pyruvate-formate lyase pathway. c. Pyruvate dehydrogenase pathway. d. Also of importance for heterofermentative bacteria in the presence of other hydrogen acceptors; acetic acid is produced rather than ethanol. TPP, thiamine pyrophosphate. (After various sources.)

   b. H_2O_2 is produced. The ratio of the activities of enzymes that form H_2O_2 and break it down determines whether or not a bacterial growth–inhibiting content of H_2O_2 is formed. Most thermophilic lactic acid bacteria accumulate H_2O_2 in milk up to 1–2 mg $\cdot$ kg^{-1}. If the peroxidase–thiocyanate–H_2O_2 system (Section 2.5) is present, H_2O_2 may act as a growth inhibitor, even though its concentration is too low for it to act as such by itself.

   c.   Oxidation of NADH to NAD$^+$ enables the bacteria to perform alternative dissimilation processes that yield energy. As a result, heterofermentative lactic acid bacteria can produce acetic acid rather than ethanol from acetyl-P, thereby doubling the ATP. The lactococci, and possibly other homofermentative lactic acid bacteria, have the pyruvate dehydrogenase enzyme that is involved in acetyl-CoA synthesis from pyruvate. During this synthesis, CO_2 and NADH are formed. Acetyl-CoA can be converted to acetic acid or ethanol. The more likely step under aerobic conditions is the production of acetic acid because it yields ATP; moreover, the enzymes of the ethanol pathway that need NADH are markedly hindered by the oxidase/peroxidase system.

     Pyruvate oxidase/NADH peroxidase action has been found in some of the lactic acid bacteria, but not in most lactococci. Pyruvate oxidase catalyzes the conversion of pyruvate to acetyl-P, which is hydrolyzed into acetic acid and ATP. A simplified pathway description is according to
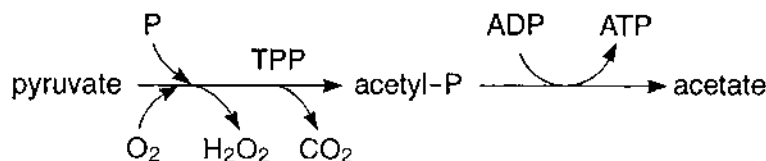
where TPP = thiamine pyrophosphate.

     (3)   An important alternative metabolic pathway is the citrate metabolism (Section 11.1.3).

     All in all, alternative breakdown processes may yield additional energy (deposited as ATP), which is used for a faster growth and an increased cell mass production. In the initial growth stages, action of pyruvate dehydrogenase in the presence of O_2 yields CO_2; such action also significantly enhances growth. Apart from that, *Streptococcus thermophilus* can hydrolyze urea to NH_3, which is assimilated, and CO_2. The organism thus shows a rapid and extensive CO_2 production during growth in milk; this property is of importance in yogurt manufacture.

11.1.2 Type of Lactic Acid Formed

The acid formed in the lactic fermentation can be the dextro-rotatory L(+) lactic acid or the levo-rotatory D(−) lactic acid. Which isomer is formed depends on the action of L- and D-lactate dehydrogenase, respectively. Certain lactic acid bacteria contain both enzymes, which often differ in activity so that the ratio between the amounts of the isomers produced may vary widely. If the ratio is 1, the product obtained is called a racemic mixture (DL). Some bacterial strains can form such a mixture because they contain lactate racemase, in addition to lactate dehydrogenase. The former enzyme can transform the one isomer to the other.

11.1.3 Citrate Metabolism

The homofermentative *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* and the heterofermentative leuconostocs, including the strains of *Leuconostoc mesenteroides* ssp. *cremoris*, metabolize citrate. It is not used as an energy source but is metabolized only in the presence of a fermentable sugar like lactose. Additional pyruvate is formed during citrate metabolism, so that more of it becomes available than is required for oxidation of NADH (released during the sugar fermentation). Accordingly, specific end products can be produced; these include acetic acid, CO_2, and ''C_4 products,'' including diacetyl, which is an important flavor compound in some milk products. The organisms involved are sometimes called aroma-forming bacteria.

Citrate is transported into the cell by citrate permease. The metabolic pathway is shown in Figure 11.2. At first, citrate is hydrolyzed into acetate, CO_2, and pyruvate by citrate lyase according to

$$COOH \cdot CH_2 \cdot C(OH)COOH \cdot CH_2 \cdot COOH \rightarrow CH_3 \cdot COOH$$
$$+ CO_2 + CH_3 \cdot CO \cdot COOH$$
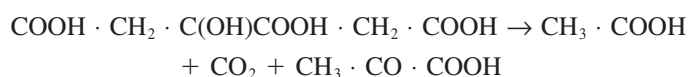
Formation of diacetyl ($CH_3 \cdot CO \cdot CO \cdot CH_3$) from pyruvate then can occur directly from active acetaldehyde and acetyl-CoA, or via α-acetolactate formed by condensation of active acetaldehyde and another molecule of pyruvate. Both reactions occur simultaneously. α-Acetolactate is decarboxylated to acetoin ($CH_3 \cdot CO \cdot CHOH \cdot CH_3$, no flavor compound); the formation of diacetyl from α-acetolactate depends on the redox equilibria in the system. α-Acetolactate is an unstable molecule and at low pH may be nonenzymatically decarboxylated to acetoin or, in the presence of oxygen, oxydatively to diacetyl. In the latter case, α-acetolactate should be extracellular; production of diacetyl via the α-acetolactate pathway appears to be most common.

During growth of *diacetylactis* strains in milk, the contents of diacetyl and

acetoin keep increasing as long as citrate is present. Citrate suppresses the synthesis of both diacetyl and acetoin reductases. Accordingly, once citrate has become exhausted, reduction in the levels of both diacetyl and acetoin occurs, by formation of acetoin and 2,3-butylene glycol ($CH_3 \cdot CHOH \cdot CHOH \cdot CH_3$), respectively. However, the pH has become low in the meantime and so have the activities of the above-mentioned reductases.

During growth of the leuconostocs in milk, the metabolism of pyruvate depends on the pH. Production of diacetyl and acetoin only occurs below pH ~ 5.5. The explanation is uncertain, but various intermediate metabolites of the heterofermentative sugar fermentation have been shown to inhibit the formation of acetolactate synthase; during the initial growth stage, the production of α-acetolactate is thereby suppressed. There is no NAD^+/NADH involved in the conversion of pyruvate to diacetyl and acetoin via α-acetolactate. Reduction of diacetyl and acetoin requires NADH. It enables the leuconostocs to oxidize NADH and to produce acetic acid (with a yield of ATP) rather than ethanol during sugar metabolism. It follows that flavor compounds are lost, to an extent that depends on the bacterial strain involved. All in all, leuconostocs have a much stronger reducing capacity than the *diacetylactis* strains.

Strains of *Streptococcus thermophilus* and of *Lactobacillus delbrueckii* ssp. *bulgaricus* cannot metabolize citrate. Therefore diacetyl and acetoin must be formed from pyruvate produced during sugar metabolism.

11.1.4 Production of Acetaldehyde

Acetaldehyde is predominantly accumulated by lactic acid bacteria that have no alcohol dehydrogenase enzyme. These bacteria therefore cannot reduce acetaldehyde (formed via the pyruvate-formate lyase pathway or the pyruvate-dehydrogenase pathway) to ethanol. Examples of acetaldehyde-accumulating bacteria are *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis, Streptococcus thermophilus*, and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The latter bacterium also produces acetaldehyde from the amino acid threonine according to:

$$\text{threonine} \xrightarrow{\text{threonine aldolase}} \text{acetaldehyde} + \text{glycine}$$
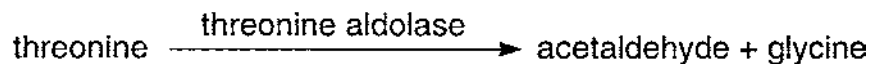
Far more acetaldehyde is produced via this pathway than via the other one.

Acetaldehyde is an important component of yogurt's aroma.

11.1.5 Growth in Milk and Stimulation of Growth

Lactic acid bacteria require many nutrients. Milk contains insufficient amounts of immediately available nitrogenous compounds (i.e., low molar mass peptides
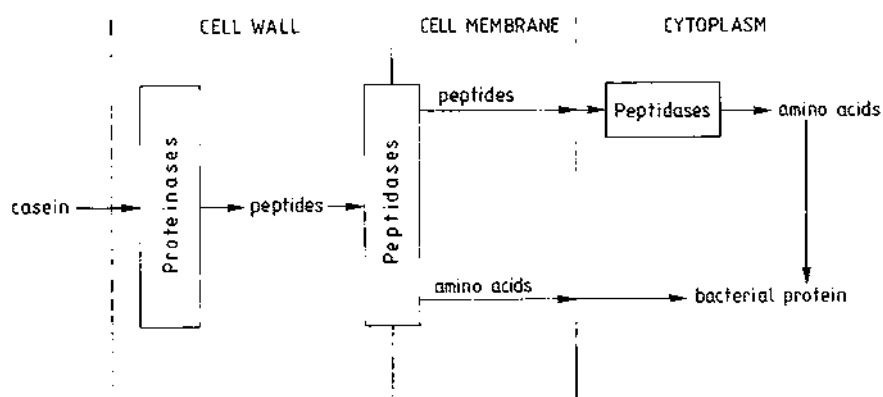
FIGURE 11.3 Degradation of casein by lactic acid bacteria in favor of bacterial growth. If the culture medium contains free amino acids, these can also enter the cell. After F.A. Exterkate, *Neth. Milk Dairy J.* **29** (1975) 303.

and amino acids) to sustain the growth of the bacteria. A prerequisite for good growth in milk is the presence in the bacterial cell of a proteolytic enzyme system consisting of enzymes associated with the cell envelope as well as intracellular enzymes. The consecutive enzymes hydrolyze the large protein molecules to assimilatable components (Fig. 11.3). Not surprisingly, the presence of this system in lactic acid bacteria on the one hand, and their growth and acid production rate on the other hand, are significantly correlated. Strains that are missing the cell wall proteinases (called prt⁻ strains) hardly grow in milk. In mixed cultures, these strains rely on the nitrogenous compounds produced by prt⁺ strains. The quantity of cell wall proteinase formed by prt⁺ strains is greatly reduced if sufficient small peptides are present in the culture medium.

Commensalism refers to one organism promoting growth of another, without benefiting itself. An example is the mentioned presence of prt⁺ lactococci enabling the growth of prt⁻ variants. When organisms can grow independently but do so better when together, this is called *mutualism* or *protocooperative growth*. A good example is the mutual growth stimulation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in yogurt (Section 20.3). Another example is the growth of *Leuconostoc mesenteroides* ssp. *cremoris* in a mixed culture. Unlike many strains of *Leuconostoc lactis*, the former leuconostoc (prt⁻) hardly grows in milk but shows satisfactory growth in a culture with prt⁺ lactococci. Conversely, *Leuconostoc mesenteroides* ssp. *cremoris* enhances the growth of the lactococci by production of CO_2.

Besides stimulation of the growth of other bacteria, inhibition may occur. This is briefly discussed in Section 11.4.3.

11.1.6 Proteolytic and Lipolytic Activities

Species and strains of lactic acid bacteria differ in their capability of hydrolyzing protein. This is partly caused by the organization of their proteolytic enzyme system. Usually, the thermophilic rods have a higher proteolytic activity than the mesophilic and thermophilic cocci. The lipolytic activity of the lactic acid bacteria is limited. It mainly concerns the hydrolysis of di- and monoglycerides formed from triglycerides by foreign lipases.

Proteolysis and lipolysis by lactic acid bacteria during ripening of cheese are discussed in Chapter 23.

11.1.7 Formation of Polysaccharides

Most strains of *Streptococcus thermophilus* and of *Lactobacillus delbrueckii* ssp. *bulgaricus* produce polysaccharides. A layer of these polysaccharides, built of galactose and other sugar residues, can envelop the bacterial cells; this is called a glycocalix. The polysaccharides can also be excreted into the medium and then are called exopolysaccharides. The substances are of importance for the properties of stirred yogurt (Section 20.3). Some strains of *Lactococcus lactis* ssp. *cremoris* and ssp. *lactis* can also produce polysaccharides (Section 20.2).

11.1.8 Plasmid-Encoded Properties

It is desirable, of course, that the metabolic properties of lactic acid bacteria are stable. However, some properties are fairly unstable. This should be ascribed to these properties being plasmid-encoded in the bacteria, which means that these properties are determined by genes that are localized in plasmid DNA. During replication, the plasmid DNA multiplies independently of the chromosomes. Several plasmids can occur in the cell. During replication, each daughter cell acquires a replica of the chromosomal DNA and, in most cases, replicas of the plasmids that occur in the cell. If the daughter cell is not provided with the replica of a certain plasmid, this cell will not show the property determined by the plasmid involved.

Several properties can be plasmid-encoded, e.g., uptake of sugar via the phosphotransferase system, synthesis of cell wall proteinases, citrate metabolism (or the permease system), cell wall resistance toward bacteriophages, production of polysaccharides, resistance to antibiotics, and susceptibility to the peroxidase–thiocyanate–H_2O_2 system. By application of genetic engineering these properties of lactic acid bacteria may in principle be stabilized.

The properties of lactic acid bacteria discussed in Section 11.1 strongly affect composition and properties of starters (Section 11.4).

11.2 ACID PRODUCTION

The prime property of lactic acid bacteria is the production of acid, especially lactic acid. Evaluation of growth then is traditionally done by determining the quantity of acid produced by titration. The titration values often are (were) expressed as percent lactic acid. This is generally not justified, as discussed in Section 1.2.2. For most applications, knowledge of the pH is of greater relevance. Moreover, pH can be measured in line, which may be of considerable advantage for monitoring or controlling acidification processes.

The relations between pH, titratable acidity, and bacterial count show several complications:

   a. *Acid dissociation.* If milk is acidified (or titrated) with HCl, the acid is fully dissociated at the pH values of interest, but this is not the case for lactic acid. The latter has a pK of about 3.9, implying that below pH $\approx$ 5.8 the acid is not fully dissociated. Moreover, lactate ions associate to some extent with Ca^{2+} and Mg^{2+} ions. Equation (2.4) allows calculation of the dissociation. Figure 11.4a, curves 1 and 2, shows the difference between HCl and lactic acid, and it is seen to be considerable at low pH.
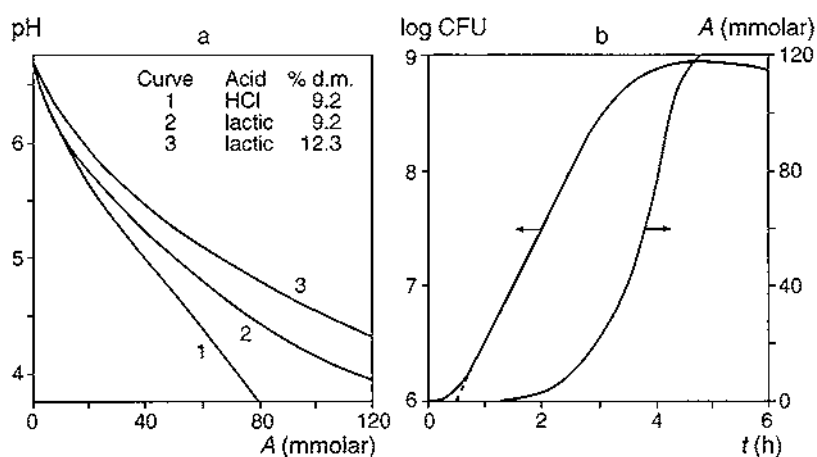
FIGURE 11.4 Acid production by starter bacteria. (a) Titration curves of plain and concentrated skim milk with hydrochloric or lactic acid; d.m. = dry matter. (b) Bacterial count (colony-forming units, in ml^{-1}) and amount of acid produced (*A*) as a function of the time (*t*) after a certain quantity of starter (roughly 0.1%) was added to the milk; constant external conditions. Approximate examples.

b. *Buffering capacity*. The quantity of acid needed to produce a given decrease in pH depends on the buffering capacity of the milk, hence on its composition. Buffering primarily is due to protein and salts. This is discussed in Section 1.2.2, and Figure 1.2 gives examples. Figure 11.4a, curves 2 and 3, shows the difference between plain and concentrated skim milk, the latter being typical for traditional yogurt. Since the buffering capacity of milk fat globules is virtually zero, the titratable acidity of a cream of $x\%$ fat will be about $(100 - x)\%$ of that of the corresponding skim milk.

c. *Other changes* in the milk due to bacterial growth may affect the relation between pH and acidity. Other acids may be formed, e.g., acetic ($pK = 4.7$) and carbonic acid (first $pK = 6.3$). Citric acid may be consumed (Section 11.1.3), thereby slightly lowering acidity. Proteolysis may somewhat enhance buffering capacity, and excessive lipolysis increases acidity.

d. *Bacterial count versus acid produced*. Figure 11.4b shows such a relation. The first thing to consider is that bacterial counts are commonly given on a log scale, whereas the rate of acid production would in first approximation be proportional to the actual number. For the simplest case, i.e., during the exponential growth phase, the relation between acid produced A (in mol/L) and incubation time t would be

$$A = cN_0 g(2^{t/g} - 1) \tag{11.1}$$

where c is a proportionality constant (in mol/s), N_0 the initial count, and g the generation time of the bacterium.

e. *Bacterial mass versus count*. Other things being equal, the acid production rate would be proportional to the bacterial mass, and the number of colony-forming units (CFU) per ml may not be proportional to this mass. Upon growth, i.e., division, lactococci generally remain associated in chains of tens of cells, and each chain produces at most one CFU. The relation between mass and CFU may vary considerably among species or strains; it may also depend on growth conditions.

f. *Bacterial metabolism* may vary considerably (see Section 11.1). Some species or strains produce four molecules of lactic acid from one molecule of lactose, others only two, and other variations occur.

g. *Decoupling* of growth and metabolism may occur. Only under fairly ideal and constant conditions, especially in the exponential growth phase, acid production rate is proportional to bacterial mass. However, when growth slows or even stops, especially due to the accumulation of lactic acid, the enzyme system of the bacteria may still go on converting lactose to lactic acid. This is illustrated in Figure 11.4b (after about 3 h). Decoupling may also occur at other conditions unfavorable

for growth, such as low temperature, high salt content, or a combination of these. At still more extreme conditions, acid production stops as well.

It may be added that it is the concentration of true lactic acid, i.e., in its undissociated state, that determines whether growth stops. However, the lower the pH, the higher the concentration of undissociated lactic acid at a given total concentration [according to Equation (2.4)]. The inhibiting effect of other acids, e.g., acetic or sorbic acids, also depends on the concentration undissociated.

The rate of acid production naturally varies greatly with growth conditions, such as temperature, pretreatment of the milk (especially heat treatment), oxygen pressure, etc. (see also Section 4.1.2). When checking the growth or acidification capacity of various starters in laboratory tests, one should ensure that these growth conditions are exactly the same as during manufacture.

When a starter is added to the milk, the growth conditions for the bacteria are often not quite the same as was the case in the starter, e.g., the heat treatment of the milk and its oxygen pressure may well be different. It generally means that the bacteria have to adapt their enzyme system to some extent before the maximum growth rate is attained. This shows up in a lag time—about 0.5 h in the example of Figure 11.4b.

11.3 BACTERIOPHAGES

Bacteriophages, or phages for short, can kill bacteria. They are not living organisms but can proliferate in a bacterial cell. This implies that they interfere with the bacterium's metabolism, so that the cell produces phages rather than its own building blocks. Phages occur in a wide variety of species, each of which acts specifically on a certain bacterium species or only on a certain strain or group of strains. Bacteriophages occur in raw milk, but generally in very low numbers. They can proliferate where bacteria are grown in large quantities, as in starters. In such a case, a bacteriophage can be very harmful in the manufacture of fermented milk products, as it can kill a vast majority of the bacteria present.

A bacteriophage is defined as a virus that can infect a bacterial cell. Infection occurs if the phage ''fits'' the cell; it is then referred to as a homologous phage. Whether or not a phage fits often depends on the bacterial strain involved. A certain phage strain can usually infect several closely related bacterial strains. The latter strains account for the ''host range'' of the phage. One bacterial strain may belong to the host range of various phage strains. Apart from the ability to infect cells, viruses differ from bacterial cells by the absence of a metabolic system (as mentioned, viruses cannot be regarded as living particles); hence, their multiplication depends on the biochemical outfit of the host cell. Furthermore, they have only one type of nucleic acid (DNA or RNA). Our knowledge of phages

is mainly taken from the so-called T phages of *Escherichia coli*. Based on that knowledge, the main differences in properties of various phages are as follows:

    a. *Differences in shape*. Phages can consist of a head and a contractile or noncontractile tail. Tailless phages also occur as well as filiforms. Most phages for lactic acid bacteria are isometric types, with a small head and a short tail (40–60 and 110–150 nm, respectively) or, not so often, with a great head and a long tail (65–85 and 450 nm, respectively). Furthermore, phages occur with an extended head (60 × 45 nm, prolate phages) and a short tail (~90 nm).

    b. *Differences in the nucleic acid* type and in the order of these acids in a single or double helix. Phages for lactic acid bacteria have DNA in a double helix.

    c. *Differences in effects* that phages bring about after infection. Virulent phages dissolve the bacterial cell, i.e., lysis. Some filiform phages leak from the cell without killing it. Other phages multiply in the cell without causing lysis, whereas still others integrate into the bacterial cell (prophage, lysogenic state; see below).

Lysis of bacterial cells is the phenomenon by which the presence of disturbing phages becomes noticeable. Lysis can be induced as follows. A suspension containing a phage and a phage-sensitive bacterial strain is put in or on a nutrient agar. After incubation, cleared sites (plaques or plages) are visible amidst a thickly grown culture. On these sites bacterial cells have been lysed. If a bacterial cell in the suspension is infected by only one phage particle (presence of a relatively small number of phages), the number of plaques reflects the number of infectious phages (plaque-forming units, or PFUs) in the suspension.

11.3.1 Phage Growth in the Bacterial Cell

The growth of a virulent phage in a bacterial cell can be monitored by enumerating the infectious phage particles in a suspension containing young bacterial cells and the phages involved. To achieve this, the suspension is plated after various times in nutrient agar, which has been inoculated with the phage-sensitive bacterial strain. The conditions in the suspension should be such that:

    a. A bacterial cell can be contaminated by only one phage particle.

    b. In the suspension free phages are absent (a free phage is called a virion). To that end, phage particles that have not become attached to a young cell during the adsorption stage and thus have not infected it must be destroyed. This is achieved by means of an antiserum, which is subsequently inactivated.

    c. The phages that are released on lysis of the infected cells cannot infect new cells. To achieve this, the suspension is markedly diluted.

> d. The bacteria can satisfactorily ''grow'' because the metabolic activity of the cells determines the multiplication of the phage.

Figure 11.5 illustrates the increase of the number of PFUs under the conditions mentioned. Three periods are distinguished:

> a. *A latent period in which the PFU number remains unchanged.* The suspension does not contain free phages and every plaque reveals an infected center, i.e., a site infected with phages released from one cell; the cell has been infected in the suspension and has been lysed during the subsequent incubation.
> b. *A period in which the PFU number increases sharply.* The increase is caused by free phages. The cells in the suspension increasingly start lysing and the released phages diffuse throughout the suspension.
> c. *A period in which, again, the PFU number remains stable.* All cells in the suspension have been lysed and the number of free phages in the suspension has reached the maximum value, i.e., the plateau value.

Obviously, a plateau is reached only after some time. This is because of variation in the individual phage–host relationships and in the time over which such a relationship is established. The burst size, i.e., the number of phages that on average are released from an infected cell can be considerable. The latent period and the burst size (for lactic acid bacteria 30–60 min and up to about 100 phages
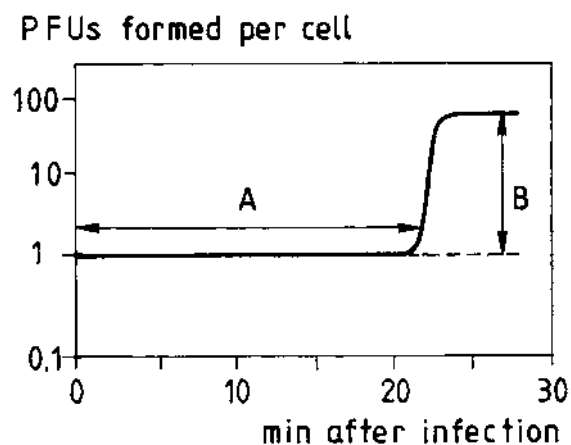
FIGURE 11.5 The growth of a phage in a bacterial cell. A = latent period; B = burst size. After J. Douglas, *Bacteriophages*, Chapman and Hall Ltd, London, 1975.

per cell, respectively) vary with the phage strain, the bacterial strain, and the physiological condition of the latter.

When phages are released repeatedly and then infect remaining bacterial cells, they soon cause culture failure.

11.3.2 Structure and Function

Structure and function of phages are illustrated by taking coli phage T2. This is not because this phage would be characteristic of other phage types but because it is most thoroughly investigated. The phage resembles an angular tadpole (Fig. 11.6). The head, shaped like an elongated bipyramidal hexagonal prism, consists of packed DNA surrounded by a protein layer. The tail is composed of a hollow cylinder (nucleus and nucleus channel) that is entirely encompassed in a contractile sheath, and a hexagonal endplate fitted with pens and long, broken fibrils.

The infection process of the phage is characterized by various steps:

    a. *Adsorption of the phage onto the bacterial cell*. In liquid media, the phage undergoes Brownian motion, with its fibrils fanning out in all
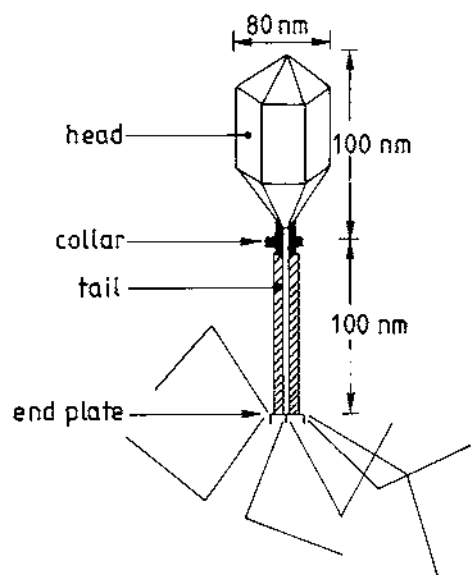
FIGURE 11.6 Structure of coli phage T2. After J. Douglas, *Bacteriophages*, Chapman and Hall Ltd, London, 1975.

directions. When the phage is in close vicinity to a host cell, its fibrils become irreversibly attached to particular cell wall compounds, e.g., lipoprotein or glycoprotein molecules. These ''receptors'' are phage-specific.

In addition to the presence of receptors, the environmental conditions largely determine the adsorption of a phage onto a bacterial cell. Inorganic salts play an important part. Many phages require bivalent cations, especially Ca^{2+} ions, to become attached to a cell. Accordingly, infection of bacteria by Ca^{2+}-requiring phages is hindered by calcium-binding compounds like EDTA, oxalate, and citrate. The acidity also plays a role: below pH 5 no adsorption occurs.

b. *Penetration of the cell wall by the phage.* When the fibrils have become attached, the endplate, in turn, sticks to the bacterial cell wall. The sheath contracts, the nucleus penetrates the cell wall, and the phage DNA gushes into the cell through the nucleus channel. A cell wall–dissolving lysozyme-like enzyme seems to be involved in the process.

c. *Intracellular growth of the phage.* After the injection of phage DNA, intricate processes alter the metabolism of the host cell in such a way that protein and DNA are formed for the phage only. These compounds are stored as separate ''precursor'' supplies. The state of the phage in the cell during the initial stage (''eclipse'') of the latent period is referred to as vegetative state. Later during that period, infectious phages are composed from the separate precursors.

d. *Lysis.* The bursting of the bacterium, which releases the phages, stops the latent period. In this process, a lytic enzyme appears to be involved, its formation being induced by the phage.

11.3.3 Cell Wall Resistance and Restriction

Combining a phage and a phage-sensitive bacterial cell does not always result in reactions as outlined above for coli phage T2. The following are among the phenomena that are responsible:

a. *Lysogeny.* This is the common phenomenon in which, after the infection by a phage, the bacteria keep multiplying in about the normal way. The phage DNA integrates into the bacterial genome and is inherited as a moderate phage or prophage by the progeny cells during their replication.

Spontaneous induction can cause the virus in a lysogenic starter to turn from a prophage into a virulent phage. Such a culture thus always contains a small number of free phages. However, no plaques are formed when the culture is plated because the lysogenic cells in such a culture are immune to further infection by the induced virulent

phage. Accordingly, the free phages in the culture can only be detected by using another, phage-sensitive indicator strain. (In an indicator strain the induced phage can multiply.)

A bacterial cell can be the carrier of more than one prophage and thereby be resistant to various phages.

b. *Pseudo-lysogeny* refers to a phage-carrying culture, i.e., a culture containing a great number of free phages. A kind of population equilibrium exists between phage-resistant and phage-sensitive cells of a strain of the culture. The latter cells account for a certain part of the population. The phages multiply in the sensitive cells, causing lysis. The released phages, however, cannot infect the phage-resistant cells and the culture keeps growing normally. A pseudo-lysogenic culture allows the bacteria to be easily separated from the phages.

To explain the pseudo-lysogeny phenomenon, phage-sensitive variants of a phage-resistant strain can be thought to be formed during propagation of the culture, e.g., because of plasmid loss. Alternatively, cells of a strain that is sensitive to a phage present in the culture may establish a physiologically determined resistance to that particular phage. In certain bacteria, the lysis of cells has been shown to cause the release of the enzyme virolysine, which damages the receptors of the other cells in the culture. Consequently, phages might not become attached to these cells. The fact that phage-carrying strains are far less sensitive to infection by phages than non-phage-carrying strains may be connected with this loss of receptors.

c. *Host-induced modification* implies that a certain phage multiplies significantly in a certain bacterial strain when it has also grown earlier in cells of the strain. It multiplies far less, however, when it has grown in another strain. This is because the previous host has modified the phage.

d. *Lysis from without*. Certain phages form lysines after having infected a bacterial cell. The lysines can destroy the cell wall of other cells, even cells that do not belong to the host range of the phage. The latter cells lyse without being infected by a homologous phage. Strains resistant to lysis have a defense mechanism and presumably have a broad phage resistance potential.

Bacterial cells may thus be resistant to phages. The resistance is based either on *cell wall resistance* (absence of receptors; the cell does not belong to the host range of the phage; phage-carrying strain; lack of factors, e.g., Ca^{2+} ions, needed for adsorption) or on *restriction* (host-induced modification, lysis from without, lysogeny).

11.3.4 Inactivation

Usual processes to destroy microorganisms (including heat treatment, disinfection, gamma and ultraviolet irradiation) can also be applied to inactivate phages, i.e., destroy their infectious ability. The phage type and the composition of the culture medium largely determine the heat resistance of phages. The inactivation is fastest in pure water and is considerably slowed down by the presence of proteins or salts, especially those of calcium and magnesium. Several phages for lactic acid bacteria survive low pasteurization of milk, i.e., 15 s at 72°C. Inactivating the most heat-resistant phages requires a heat treatment of at least 1 min at 95°C.

Several disinfectants also inactivate phages. This holds for hypochloric acid: concentration of available chlorine, temperature, contact time and type of phage strains involved determine the effectiveness of the disinfectant. Many bactericides have no effect on the inactivation of phages. These include:

- Metabolic toxins; phages have no metabolism.
- Solvents for fat like ether and chloroform. Most of the phages do not contain lipids.
- Antibiotics.

11.4 STARTERS

A starter is a culture of one or more types or strains of lactic acid bacteria that is added to milk to ferment it. Sometimes the inoculum also contains non–lactic acid bacteria, whereas in other cases the latter are added separately to the milk (see Table 11.3).

Traditionally, a starter is obtained via growth of lactic acid bacteria in milk at a suitable temperature. The starter is subsequently maintained by propagating and growing it in a fresh portion of milk. Currently, special growth media rather than milk are also utilized to avoid multiplication of bacteriophages during starter manufacture (see Section 11.4.5).

11.4.1 Composition

Table 11.3 gives a survey of the composition of starters as used in the manufacture of some fermented dairy products. On the basis of their composition, starters can also be classified as:

1. *Single-strain*. Every starter consists of a pure culture of one strain.
2. *Multiple-strain*. These consist of a defined mixture of pure cultures of

TABLE 11.3 Organisms Present in Various Types of Starter and Their Use in the Manufacture of Fermented Milk Products

| | Mesophilic | | | | Thermophilic | |
| | Aromatic | | | Nonaromatic | | |
Organism	L	DL	D	O	In cheese	In soured milks
Lactococcus lactis ssp. *lactis*	+	+	+	+		
Lactococcus lactis ssp. *cremoris*	+	+	+	+		
Lactococcus lactis ssp. *lactis* biovar. *diacetylactis*		+	+			
Leuconostoc cremoris/Leuconostoc lactis	+	+				
Streptococcus thermophilus					+	+
Lactobacillus delbrueckii ssp. *bulgaricus*					+	+
Lactobacillus helveticus					+	
Lactobacillus delbrueckii ssp. *lactis*					+	
Lactobacillus acidophilus						+[a]
Applied in						
Butter	+	+				
Buttermilk, etc.	+	+				
Sour cream	+	+				
Yogurt						+
Fresh cheese	+	+		+[b]		
Gouda-type cheeses	+	+	+	+		
Cheddar-type cheeses[c]					+	
Emmentaler[d]					+	

N.B. Kefir and kumiss (Section 20.2) have a combined lactic acid and alcohol fermentation; various kinds of lactic acid bacteria and lactose fermenting yeasts are involved. In the fermentation of varieties of cheese with a specific surface or internal flora, molds and coryneform bacteria also play a part (see Section 25.6).

[a] Only for acidophilus milk and for some types of special yogurt (Section 20.2).

[b] Used in the manufacture of cottage cheese (Section 25.2.2). The production of CO_2 by aromatic starters may cause undesirable phenomena ("floating curd").

[c] Cheese from which eyes are absent (blind cheese).

[d] Cheese varieties in which high temperatures are applied during the manufacture. In the manufacture of Emmentaler-type cheeses, the milk is also inoculated with propionic acid bacteria.

2. a few (say, six) strains of different types of bacteria or of different strains of one type. The preponderance of each strain can change.
3. *Mixed-strain*. These are natural starters, consisting of an undefined mixture of strains of different types of bacteria. The composition of this type of starter is based on a dynamic equilibrium between various starter bacteria, and it can change more considerably during use.

In mesophilic starters (Table 11.3), a single-strain starter consists of a strain of *Lactococcus lactis* ssp. *cremoris* or, less frequently, a strain of *Lactococcus lactis* ssp. *lactis* or its biovariant *diacetylactis*. Multiple-strain cultures contain strains of *Lactococcus lactis* ssp. *cremoris* and/or *lactis*, often combined with the biovariant *diacetylactis* or with *Leuconostoc cremoris* and *L. lactis*. The mixed cultures are categorized as O, L, DL, and D types; L refers to leuconostocs and D to diacetylactis being present; in an O type, neither of these two groups occur, and DL contains both.

The starter bacteria greatly affect the properties of the final product. Because of this, dairy manufacturers have increasingly focused on (a) selecting bacterial strains with desirable properties, (b) composing starters by applying suitable strains as well as appropriate proportions of these in the starter, and (c) maintaining starter composition. In doing so, new products may be developed, manufacturing processes optimized, and product properties improved.

11.4.2 Properties

The biochemical conversion of milk components by lactic acid bacteria naturally causes changes in the fermented products. These changes closely depend on the properties of the starter bacteria involved (Table 11.3) and on the type of product made. The following are the main aspects.

1. *Production of acid from lactose*. The production affects:
   a. The *preservation* of the product. The acid produced (especially lactic acid), combined with the low pH, is an essential factor for shelf life and safety of all fermented milk products. Additional factors are discussed in Sections 20.1 and 21.1.
   b. The *texture* of the product. The pH considerably affects these properties (see Sections 20.3.3 and 23.6).
   c. The *flavor* of the product (see Chapter 20 and Section 23.5).
2. *Formation of other compounds* during the fermentation of lactose and citric acid.
   a. *Flavor compounds*. It involves several metabolites, diacetyl in particular. Its formation is essential for the flavor of such products as butter made of cultured cream, buttermilk, and fresh cheese. Accordingly, these products should be made by using aromatic starters. The desired types of aroma

bacteria present in such starters may vary. For instance, a D starter is unsuitable for the manufacture of butter, since biovar. *diacetylactis* forms an excess of acetaldehyde, causing an undesirable yogurt-like flavor. A DL starter can be used, at least when the aroma bacteria involved occur in the proper ratio; acetaldehyde, formed by biovar. *diacetylactis*, then is converted to ethanol by alcohol dehydrogenase activity of the leuconostocs (see Section 11.1). Diacetyl is less important for the flavor of ripened cheese; other compounds, especially degradation products of protein and fat, can be of great importance (see Section 23.5).

In aromatic starters, the ratios between the bacterial strains involved are very critical with respect to the formation of diacetyl from citric acid. The initial number of aroma bacteria in the starter should not be too low because a rapid growth and acid production of strains of *Lactococcus lactis* ssp. *lactis* and *cremoris* would result in a low final number of aroma bacteria in the product, hence in insufficient diacetyl production. On the other hand, the number of leuconostocs in L and DL starters should not be too high. After all, these bacteria form diacetyl only at low pH. Therefore, a rapid conversion of citric acid at higher pH (to nonaromatic compounds) is undesirable. Moreover, at high pH there is an increased risk of a strong reduction of the diacetyl formed. The rate of reduction depends not only on the strain involved but on the rate of the citrate degradation. At low pH, citrate is degraded more slowly and the diacetyl reduction is less because then the lactose is also hydrolyzed more slowly (see Section 11.1).

b. *Carbon dioxide*. The production of CO_2 by aroma bacteria is essential for the texture of cheese in which the formation of a few ''eyes'' is desirable, as in Gouda and Edam cheese. Originally, these bacteria had to be absent in starters used for the manufacture of cheese with a close texture, e.g., Cheddar-type cheeses. Presently this requirement is less rigid because the cheese can be pressed under vacuum, which usually prevents eye formation (see Section 25.5).

c. *Bacterial exopolysaccharides*. The consistency of stirred yogurt greatly depends on the strains of the bacteria used, and the exopolysaccharides produced by them are held to be responsible (see Section 20.3.3).

3. *Proteolysis*. Protein degradation affects:

   a. The *consistency* of the product. The effect of proteolysis on the consistency of cheese is discussed in Section 23.6.

   b. The *flavor* of the product. Section 23.5 deals with the importance of proteolysis for the flavor of cheese. Another example is the formation of the aroma compound acetaldehyde from threonine in yogurt (Section 20.3.1; see also Section 11.1.4). The degradation of protein is essential for ripened varieties of cheese. For fresh cheese and products like butter and fermented milks, proteolysis during storage usually is undesirable.

c. The *mutual growth stimulation* of lactic acid bacteria (commensalism and mutualism; see Section 11.1).
4. *Lipolysis*. The formation of fatty acids is important for the flavor of ripened cheeses. Hydrolysis of fat during storage is undesirable for products that are consumed shortly after manufacture. Most of the lactic acid bacteria cannot hydrolyze triglycerides, but they can mono- and diglycerides; hence, they can enhance ongoing hydrolysis of fat.

In conclusion, the conversions by lactic acid bacteria strongly determine shelf life, safety, consistency, and development of flavor and texture of fermented products, including cheese. Moreover, they may affect the nutritional value (see Sections 20.1.2 and 23.8). The properties of the bacterial strains present in a starter determine the extent to which the starter can contribute to any of the product variables mentioned. In other words, the selection of a starter must be based on the properties desired in the product to be made.

11.4.3 Shifts in the Flora

The properties of a fermented milk product should for the most part be constant and stable. To fulfill this condition, starters of constant activity should be used. In other words, the ratios between the numbers of the various bacterial strains in the starter and the genetic properties of these strains should not vary. Ensuring constant bacterial properties is not easy, especially in mixed-strain starters. Several factors can be responsible for shifts in the bacterial population when the starter is propagated in the traditional way. Important are:

1. Factors concerning the *composition of the medium* (see also Section 4.1.2), including
   a. Presence of compounds slowing down or preventing growth of a bacterial strain in milk. We distinguish:
      • Natural inhibitors, especially agglutinins and the lactoperoxidase–CNS–H_2O_2–system (see Sections 2.4.3 and 2.5.2). Their concentrations strongly depend on the heat treatment of the milk. This is discussed in Chapter 6 (see especially Figure 6.14).
      • Added inhibitors, e.g., antibiotics, disinfectants.
      • Inhibitors formed by some strains, e.g., hydrogen peroxide and compounds such as nisin, diplococcin, and other bacteriocins, that may show antibiotic action against other strains in the starter.
      • Free fatty acids. Fairly low concentrations of low molar mass fatty acids (C_4–C_{12}) and of oleic acid may slow the growth of some strains.
   b. Concentration of CO_2 and other growth-promoting substances, e.g., those formed during heat treatment of milk.
   c. Concentration of trace elements. Of main importance for growth are Fe^{2+},

Mg^{2+}, Se^{2+}, and Mn^{2+}. The manganese content determines the growth of *Leuconostoc cremoris*. The organism grows poorly at a low Mn^{2+} content in milk, which may cause a starter to turn from the DL type to the D type. The situation is reversed at a high Mn^{2+} content, i.e., a DL starter can become an L starter.

   d. Concentration of O_2. Excessive concentrations are toxic for growth. At lower concentrations the effect on the growth varies and depends on the bacterial strain involved; see Section 11.1.

2. *Contamination* of the milk by:
   a. Bacteriophages. The phages can upset the composition of the starter culture severely and even destroy the starter; see Sections 11.3 and 11.4.5.
   b. Other lactic acid bacteria or other microorganisms.

3. *Mutants* with changed properties can form, e.g., because of the loss of plasmid-coded properties. Mutation can also lead to strains with an increased acid production rate.

4. *Mutual growth promotion* of starter bacteria can cause great changes in the ratio of the numbers of the various bacterial strains (see Sections 11.1 and 20.3.1).

5. *Incubation conditions* of the starter. Important are:
   a. Incubation temperature. The equilibrium between bacterial strains in a culture is determined by their relative growth rate or by their survival at the incubation temperature.
   b. Inoculum percentage. This factor can have a considerable effect on the composition of the flora, as is clearly shown for the yogurt bacteria (Section 20.3.1).
   c. Stage of growth of the starter. During propagation of the starter the ratios of the numbers of the bacterial strains keep changing. The composition of the starter culture is in each case determined by:
      • The growth rate of any of the strains as determined by genetic properties and growth conditions; see the preceding points.
      • The different susceptibility of the strains to pH and to metabolites, lactic acid in particular. It causes the growth of various strains to be slowed down to different extents. The bacteria die when the starter is incubated for too long a time (high lactic acid content, low pH, etc.). The composition of the starter then is increasingly determined by the longest surviving strains.

Several of the above-mentioned factors are just as important if pure cultures are used to propagate the starters.

   A starter of optimum composition does not suffice for a satisfactory process control. A constant day-to-day rate of acid production is important. In cheese making, for instance, a change in this rate considerably affects the syneresis rate of the curd and thereby the water content of the cheese (see Section 22.2). During manufacture, therefore, the conditions determining growth and activity of the bacteria should remain the same.

Furthermore, the rate of acid production, as partly determined by type and genetic properties of the starter bacteria (slow or rapid strains may be involved) and by inoculum percentage, may change during manufacture. Several of the above-mentioned factors have an effect, the most important being contamination by bacteriophages. The processing conditions are also essential. For example, the rate of acid production can be decreased by NaCl or by a heat treatment just below the intensity needed for killing the starter bacteria.

11.4.4 Aspects of Traditional Starter Manufacture

Traditionally, a starter is propagated by adding an amount of grown culture to a small quantity of intensely heated skim milk. The inoculated milk is incubated until a certain low pH is reached and then cooled. Usually, the ultimate pH is about 4.4. Cooling to below 5°C can prevent changes in properties of a starter for about 36 h at most. Keeping the starter without sufficient cooling decreases its acid-producing activity; part of the bacteria die when an excessive amount of acid is produced and the ratio between the numbers of the various strains may shift, at least in mixed-strain starters (see Section 11.4.3).

Every day, part of the ripe starter is inoculated in a small amount of heated milk and incubated. This is the mother culture, which is also used as an inoculum for the preparation of the bulk starter. The latter is the inoculum for the manufacture of the product on the next day (see Fig. 11.7). The milk used for the bulk starter is batch-pasteurized, e.g., 10 min at 90°C, or heated by flow-through pasteurization, e.g., 1 min at 95°C. The required amount of mother culture is determined by the quantity of product that is to be made and by the inoculum percentage. For some products, e.g., cultured buttermilk, the bulk starter may be the end product.

Intense heating of the milk used for starter preparation kills undesirable organisms, inactivates growth inhibitors and bacteriophages, and forms growth-promoting substances. A heating intensity corresponding to at least 1 min at 95°C is needed to destroy the most heat-resistant bacteriophages. Contamination by disturbing bacteriophages (Section 11.4.5) can, however, not be fully prevented by using traditional propagation methods.

Mesophilic starters are incubated at about 20°C for about 20 h; the inoculum percentage is 0.5–1. Thermophilic starters are incubated at 40–45°C for a few hours, after adding a small percentage of inoculum. To preserve the properties of the starter, mother culture and bulk starter should be prepared under identical conditions, even from day to day. This requires constant incubation time and temperature, inoculum percentage and acidity of the ripe starter, and a fairly constant composition of the milk. The milk should not be contaminated by effective concentrations of inhibitors, antibiotics in particular. Special milk powders are available to make starter milk. Even at the best of times, the traditional method
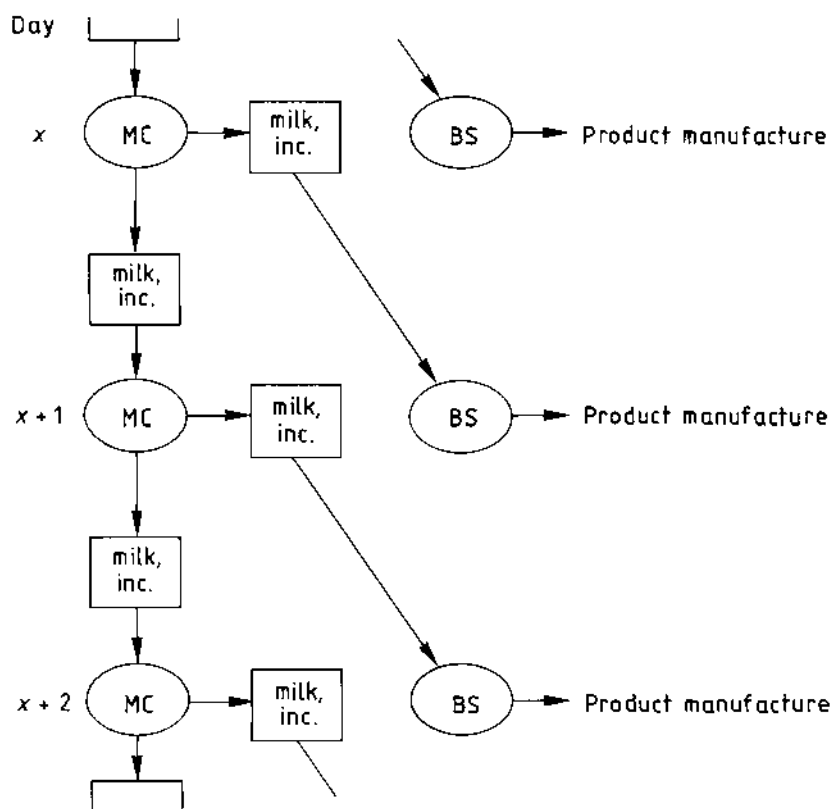
FIGURE 11.7   Propagation of a mother culture (MC) and preparation of a bulk starter (BS) for consecutive days (*x*, *x* + 1, etc.) of production. milk = starter milk; inc. = incubation.

for starter preparation does not guarantee constant composition and properties of the starter (see below).

11.4.5   Applications in the Dairy Industry

11.4.5.1   Traditional Propagation of Single-Strain Mesophilic Starters

Single-strain starters or combinations of these, propagated in the traditional way (Section 11.4.4), were until 1980 often used for Cheddar cheese. Initially, in each

plant cheese was made from day to day by using the same homofermentative *Lactococcus lactis* strain (isolated from a mixed strain starter), which was aseptically propagated. However, these strains were highly phage-sensitive and measures taken to avoid contamination by homologous lytic bacteriophages often failed. This became manifest when cheese making plants became large: the production of several consecutive batches of cheese on one day readily caused bacteriophages to accumulate in the plant. Figure 11.8 illustrates the effect of a homologous phage attacking a single-strain starter, inoculated in milk. Only a small part of the bacterial culture is phage-resistant (in the example 0.01%). When a normal inoculum percentage is used, resulting in about 10^7 bacteria per ml inoculated milk, the number of cells drops to 10^3 per ml; thereafter, it increases again due to the growth of resistant variants. Under normal incubation conditions, say
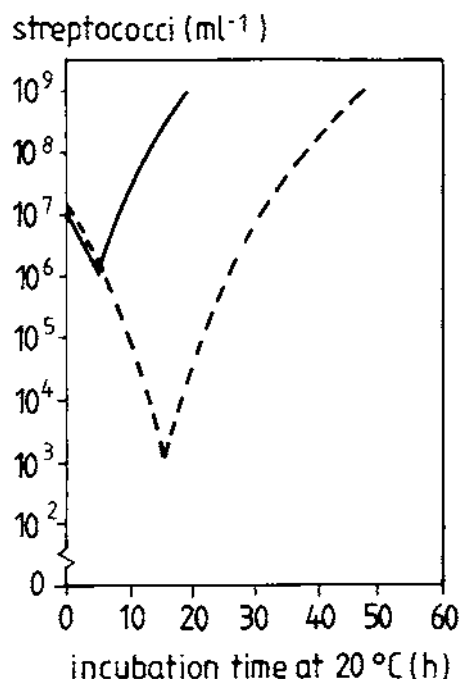
FIGURE 11.8 Behavior of two types of starter after being attacked by a disturbing bacteriophage. Solid line: behavior of a P starter (contains 10% resistant strains); broken line: behavior of a single-strain starter (0.01% resistant variants). In both instances 1% starter was added to milk. After J. Stadhouders and G.J.M. Leenders, *Neth. Milk Dairy J.* **38** (1984) 157.

20 h at 20°C, these variants do not grow to 10^9 cells per ml starter, needed to ascertain a satisfactory rate of acid production. In other words, the starter is unfit for use.

To solve these problems, traditionally propagated single-strain, non-phage-related cultures were introduced, which were applied by rotation. Moreover, use of a starter containing two strains with different properties was found necessary to make Cheddar cheese of a satisfactory quality, in particular to prevent the bitterness defect. Mother cultures of each strain were propagated, maintaining strictly hygienic conditions. For instance, strains A + B were used on the first day, C + D on the second, etc., and after a long interruption again A + B. The basic idea of this system was that strain A might be exclusively contaminated by a homologous bacteriophage a, strain B by a homologous phage b, etc. In other words, strain B would be ''non-phage-related'' to strain A. Furthermore, during the long period between the times the same strains were used, accumulation of disturbing phages would be avoided by an intervening cleaning of the equipment and by absence of host cells, needed for multiplication of the phages. The system thus was implicitly based on the idea that the genetic properties of the bacterial strains with respect to their resistance to phages, and the properties of the phages with respect to their host range, are invariable. It is now clear that this idea was incorrect and that thereby the rotation system can lead to problems (see Section 11.4.5.3).

11.4.5.2 Traditionally Propagated Mixed-Strain Starters

These starters are of old applied in western European countries. At present they are still being used, though to a decreasing extent. Inoculated and subsequently acidified whey, still being used in certain cheese varieties, can also be regarded as a mixed-strain starter.

The starters are propagated in milk under hygienic conditions, but there is no safeguard against contamination of mother cultures and bulk starters by bacteriophages. Accordingly, the starters contain a great number of phages, about 10^8 per ml, which must be considered remainders of phage attacks. Starters propagated in actual practice (P starters), however, display a marked phage resistance, mainly caused by their phage carrying character (see Section 11.3.3). A relatively high number of starter strains, e.g., 10% of all the strains present in the starter, survives an attack of disturbing phages. Usually, such an attack goes unnoticed: the flora shifts to the resistant strains which, under normal incubation conditions (for a mesophilic starter 20 h at 20°C, 1% starter added), can grow to a number of about 10^9 per ml starter (see Fig. 11.8). The starter may show a normal rate of acid production.

In actual practice, the rate of acid production may fluctuate, especially in mesophilic starters. Judging by its acid-producing ability, the propagated starter may seem unaffected after a first phage attack, but the resultant flora now contains

an increased proportion of strains that are resistant to the ''first'' phage. A renewed attack by other phages may damage a larger proportion of the population, causing the rate of acid production to decrease further. In turn, more transfers are needed to attain the original level of the acid production rate. Recurrence of the incident intensifies the effect (see Fig. 11.9, curve 1).

Furthermore, formation of genetic variants in the starter may contribute appreciably to variations in the rate of acid production. Therefore, though disturbing phages do not surface, the flora can shift, e.g., to mutants that grow faster. Under practical conditions the mutants are destroyed soon, again due to contamination by homologous phages (periodic selection). When, however, a P starter is propagated with complete phage protection for a long time in a laboratory, it will eventually be made up of mutants that are highly phage-sensitive; it becomes a ''lab starter.'' Consequently, such a starter is rapidly destroyed when it is reintroduced under practical conditions (see Fig. 11.9, curve 2).
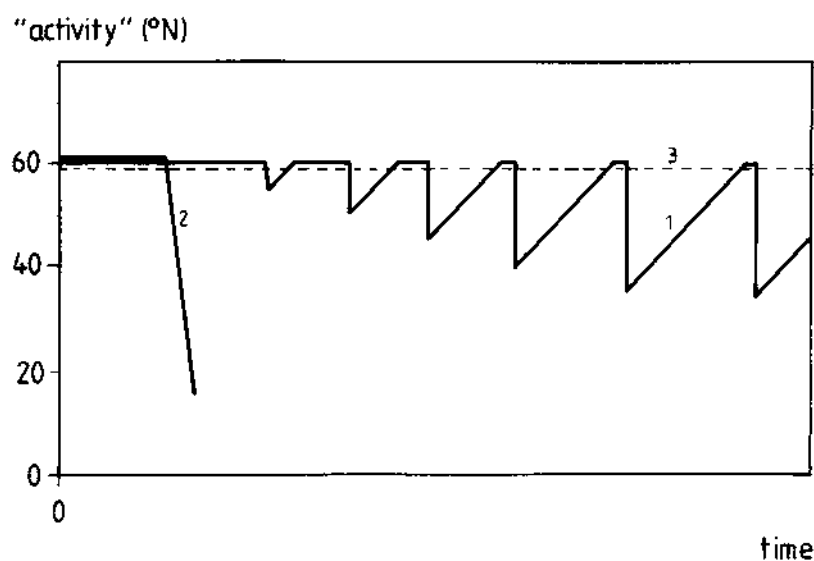
FIGURE **11.9** Fluctuations of the rate of acid production in milk by lab and P starters after introduction into a plant. 1: P starter, propagated in the traditional way; 2: lab starter, propagated in the traditional way; 3: P starter, introduced as a concentrated mother culture, while the bulk starter is cultivated under complete phage protection. The rate of acid production is expressed as the acidity of the milk, inoculated with 1% starter, obtained after 6 h at 30°C. After J. Stadhouders and G.J.M. Leenders, *Neth. Milk Dairy J.* **38** (1984) 157.

All in all, in actual practice the propagation of mixed-strain starters can be employed without too many problems. A complete drop out of the starter does not occur because resistant strains are always left after a phage attack. However, as stated before, the rate of acid production by the starter fluctuates and it can even drop to below the level desirable for product manufacture; furthermore, the composition of the starter keeps shifting. These properties do not fit the prerequisites for process control in modern cheese manufacture.

11.4.5.3   Modern Methods

In the traditional propagation of starters, mutations, such as those caused by plasmid loss, are responsible for a continuous formation of variants with altered genetic properties, including the phage sensitivity range. That means that a mutant can become a member of the host range of a particular phage or a cluster of phages to which its ''ancestor'' was impervious. On top of that, neither the pool of phages in the plant nor their genetic properties is constant. Different kinds of phages are introduced through the raw milk supply; depending on their number, virulence, and the number of host cells in the milk, they can multiply and be a danger to the starter. Incidentally, several phages survive low pasteurization of milk as applied, for instance, in cheese making. Still other phage types are accumulated in the plant by release of temperate phages from lysogenic bacterial cells. Alternatively, throughout multiplication of phages in host cells, their genetic properties can change by mutation, host-induced modification, and recombination of, say, a lytic phage with a prophage. Figure 11.10 illustrates sources of phages in a cheese plant.

From the above, the risk of contamination by disturbing phages can be inferred to be small if:

a. The supply of phages with the raw milk is small. This implies that the milk should be of satisfactory bacteriological quality. For cold-stored farm bulk milk, these requirements are usually met.
b. The genetic properties of the starter bacteria are largely fixed. This can be achieved by rapid freezing of the culture and keeping it at a temperature below $-35°C$.
c. Starter bacteria are selected that are resistant to a great number of phages (see below).
d. The number of bacterial strains in the starter is small.
e. During propagation of the starter, contamination by phages is prevented by complete protection from phage development and by frequent rigorous cleaning and disinfection during product manufacture.

Based on these considerations, a system has been set up (for the first time in New Zealand) that starts from deep-frozen, phage-resistant bacterial strains. Strains isolated from mixed-strain starters (after all, these starters are a satisfac-
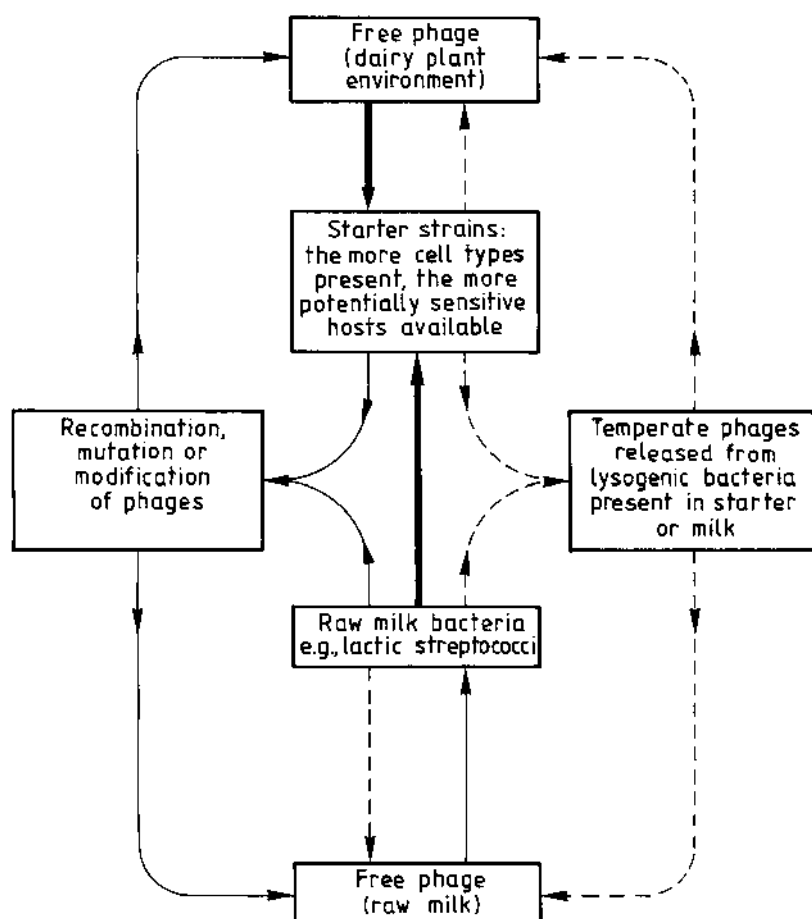
FIGURE 11.10 Sources of bacteriophages in a dairy plant. Heavy lines indicate pathways that are especially important, broken lines occasional pathways. After H.A. Heap and R.C. Lawrence (In: R.K. Robinson, ed.: *Developments in Food Microbiology*, Vol. 4, Elsevier Appl. Sci., London, 1988).

tory source of resistant strains) are tested for their resistance against a great number of phages present in the cheese whey of various plants. Subsequently, the fully resistant strains are selected on the basis of their suitability for product manufacture, i.e., rate of acid production, flavor development, resistance to antibiotics, etc. In specialized plants, the suitable strains and their mixtures are cultivated under complete phage protection, then concentrated and distributed while

deep-frozen. (To avoid cell damage caused by the freezing procedure, lactose is added to the concentrate as a cryoprotectant.) The concentrate can be stored for several months without appreciable loss of properties. Obviously, by means of selected strains all kinds of starters can be composed, nonaromatic, or aromatic mesophilic, as well as thermophilic starters.

In the dairy plant, the concentrate represents a mother culture with constant bacterial composition and acid production rate; it serves to prepare the bulk starter culture. Every day the bulk starter milk is inoculated with a new unit of thawed concentrate. It is propagated with complete protection from phage development, which requires special provisions for the fermentor, including a phage filter and a device for inoculation of the bulk starter milk. Applying closed machinery (e.g., closed curd-making machines) and maintaining a satisfactory hygienic standard serve to minimize contamination with phages and their accumulation during product manufacture. In practice, the starters can be used for a long time in succession; a rotation system is not needed. Apart from that, when a disturbing phage surfaces, the phage-sensitive strain can be substituted by a resistant one. The number of strains in the starter is restricted to a maximum of six; this is called a multiple-starter system. Sometimes not more than two strains are used (single-pair system).

An older system (developed in The Netherlands) is based on the use of concentrated mother cultures consisting of P starters. Otherwise the two systems do not differ essentially. Mixed-strain starters of a satisfactory acid production rate, fit for making good-quality products, are obtained from various dairy plants. Every starter is immediately deep-frozen to fix the composition of bacteria and phages, along with the properties of the starter, including its natural phage resistance. The starters are rarely transferred. In producing concentrated mother cultures, the proportions of the various strains in the starter should be maintained, especially the ratio of aroma-forming to non-aroma-forming bacteria. Maintaining the proper ratio is easiest when the starter is cultivated at a nonconstant pH. The ripe starter is neutralized with alkali, then concentrated by bactofugation to a concentration of bacteria about 40 times that in a conventional starter; for thermophilic (yogurt) cultures this is about 20 times. After lactose has been added, the product is deep-frozen and transferred to the users.

Again, the latter system ensures a constant bacterial composition of the mother culture as well as an almost complete elimination of fluctuations of the starter activity during product manufacture (see also curve 3 in Fig. 11.9). The system is used for cheese as well as for other products.

If the above-mentioned systems are applied, contamination by disturbing phages can only occur during product manufacture. In cheese making, phage multiplication is limited because the clotting of the milk hinders diffusion of the phage through the gel. Moreover, phage accumulation in the plant can be avoided by frequent cleaning and disinfection.

Thermophilic starters cause far smaller problems than mesophilic starters; the reason is not quite clear. The phages may proliferate at a slow rate and they may have a restricted host range. For instance, *Lactobacillus delbrueckii* ssp. *bulgaricus* is insensitive to phages that attack *Streptococcus thermophilus*. Furthermore, the kind of product may be involved. For example, in yogurt manufacture no whey is released, whey being the main pool of phages and the main cause of phage accumulation in cheese plants.

Some other systems are as follows:

1. The use of *phage-resistant mutants*. Here always the same strain is used, which on a phage attack is substituted by a mutant of that strain that is resistant to the phage involved. The system should be set up for each individual plant. Mutants are obtained by a constant exposure of a growing culture of the strain to the cheese whey, i.e., to the pool of phages in the plant involved. Isolated strains are selected on the basis of their suitability for product manufacture. A drawback is that the use of one strain often causes off-flavors and, moreover, the isolation of fast acid–producing mutants having a satisfactory phage resistance is not always easy.

2. A similar way of working in starter manufacture as mentioned in point 1 is now applied in many U.S. cheese plants. A multiple-strain starter, consisting of some three to six *Lactococcus* strains, is used. Once a phage that is virulent for one of the strains becomes manifest, the strain involved is displaced by a suitable phage-resistant mutant. In most cases the starter is propagated and grown in blends of skim milk (and/ or whey), autolysed yeast, and Ca^{2+}-binding phosphates (e.g., mono- and disodium phosphate). Phages that need Ca^{2+} ions for their adsorption onto the host cell then cannot proliferate. In these ''phage inhibitory media'' the starter is grown at a constant pH of about 6. In these conditions, the starter produces acid faster than when it is grown in, e.g., skim milk without pH control. The acid production rate does not decrease rapidly while the starter is being kept at the incubation temperature. Therefore, there is less need to cool the ripe starter (see also Section 11.4.4). Not all of these substrates are equally effective and, moreover, Ca^{2+}-insensitive phages occur. Furthermore, the substrates prevent multiplication of phages during bulk starter preparation, but not during product manufacture. Accordingly, in actual practice not all problems are overcome.

3. Mixed-strain starters used by rotation. The starters are propagated with phage protection, but apart from that there is a traditional way of working. This increases the risk of producing phage-sensitive mutants, as

well as of the release of more temperate phages and of the presence of more indicator strains, caused by the great number of strains involved. Accordingly, there is an increased risk of disturbing phages.

11.4.5.4 Direct Vat Inoculation

Direct inoculation of cheese milk with an adequate number of lactic acid bacteria by means of a deep-frozen or lyophilized concentrated bulk starter culture dispenses with the preparation of a bulk starter. Because there are no propagation steps, the risk of phage attacks is minimized. The direct inoculation method has, however, little advantage over the modern starter preparation systems, which also guarantee a satisfactory control of the phage problem. A great disadvantage is the high price, so that application should only be considered if there are serious phage problems, if these problems cannot be settled by modernizing the starter preparation, and if the percentage of starter added is small (as in the manufacture of cheese varieties where very high scalding temperatures are used).

SUGGESTED LITERATURE

- There have been several symposia on lactic acid bacteria. Much general information is given in the newest report, i.e.,

    Lactic Acid Bacteria: Genetics, Metabolism and Applications, G. Venema, J.H.J. Huis in 't Veld, and J. Hugenholtz, eds., Proceedings of the fifth Symposium held in Veldhoven, The Netherlands, 8–12 September 1996.

- Much information is also in the book:

    B.A. Law, ed., *Microbiology and Biochemistry of Cheese and Fermented Milks*, 2nd ed., Blackie, London, 1997, especially Chapters 2 (classification and identification of bacteria important in the manufacture of cheese), 9 (proteolytic systems of dairy lactic acid bacteria), and 10 (molecular genetics of dairy lactic acid bacteria).

- Bacteriophages are discussed in:

    Practical Phage Control, Bulletin of the International Dairy Federation No 263, Brussels, 1991.

- See also:

    T.M. Cogan and C. Hill, Chapter 6, in: P.F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology*, Vol. 1, *General Aspects*, 2nd ed., Chapman and Hall, London, 1993.

- A comprehensive review on phages in starter organisms is:

    G.E. Allison and T.R. Klaenhammer, *Int. Dairy J. 8,* 1998, 207–226.

12

Fouling, Cleaning, and Disinfection

In the dairy industry, cleaning and disinfection are essential operations. Fouling occurs because milk residues remain on the surfaces of equipment. Residues of milk that have dried up are difficult to remove. Excessive fouling is costly because milk is lost, increased concentrations of detergents are required, and consequently more wastewater is produced. Fouling of membranes by the formation of a gel layer considerably reduces the flux (see Section 9.4). Heating of milk results in the formation of a deposit on metal surfaces that is difficult to remove. Deposit formation reduces the rate of heat transfer and the flow rate of milk in the equipment. Eventually, the equipment will stop operating. In a multiple-effect evaporator with, e.g., six effects, the costs due to fouling (milk losses + cleaning) can account for more than half of the total running costs (including machinery, energy, and so forth). Cleaning of equipment is necessary to reduce all of these problems and to prevent the growth of microorganisms in milk residues, which is highly undesirable. Several microbes can readily grow at surfaces containing a thin film of milk deposit.

12.1 DEPOSIT FORMATION

Fouling of a surface always starts with adsorption, for the most part of proteins. From milk and its derivatives first of all serum proteins may adsorb onto a metal surface, whether that surface is hydrophilic or hydrophobic. Adsorption occurs almost instantaneously and is not fouling; it concerns a monomolecular layer. However, onto this adsorbed layer more serum protein, as well as other materials, including calcium phosphates, casein micelles, fat globules, and bacteria (often by means of their glycocalix), are deposited. Figure 12.1 illustrates the difference
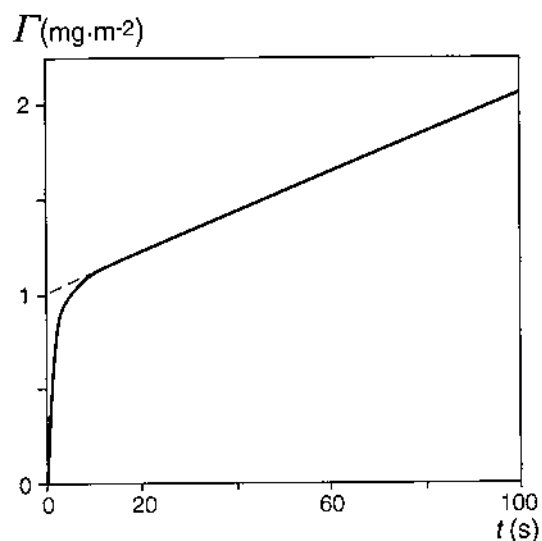
FIGURE 12.1 Deposition of protein from a very dilute solution of serum proteins onto a chromium oxide surface at 85°C. Γ = protein load, t = time. Approximate results, derived from experiments by T. Jeurnink et al., *Colloids and Surfaces B: Biointerfaces* **6** (1996) 291–307.

between the initial fast adsorption of protein and the subsequent much slower deposition.

It may be useful to give some ideas about the quantities involved. Adsorbed, i.e., monomolecular, protein layers have surface loads for the most part varying from 1 to 3 mg $\cdot$ m^{-2}. A deposit layer of 1-mm thickness, consisting of 20% protein, would correspond to a protein load of about 200 g $\cdot$ m^{-2}, i.e., 10^5 times as much. A reasonable value for the rate of deposit formation in a heat exchanger when pasteurizing milk would be 30 g $\cdot$ m^{-2} $\cdot$ h^{-1}. It would then take about 7 h for a 1-mm layer to form. In some situations, faster deposition rates are observed (see below).

Two types of deposited material are often distinguished, arbitrarily called A and B. Type A is typically formed at moderate temperatures, say 80°C. It consists, for instance, of 35% ''ash'' and 50% protein in the dry matter, and it looks yellowish, voluminous, and curd-like. Table 12.1 gives an example of its composition compared to that of skim milk. It is seen that about 10% of the deposited dry matter is not accounted for; it may be that this concerns Maillard products, etc. Type B deposit is typical for heating at high temperatures, e.g., >100°C. It contains more than 70% ''ash'' (for the most part calcium phosphate) and some protein. It looks compact, gritty, and grayish, and it is also called milk-

TABLE 12.1 Composition of the Dry Matter
of Skim Milk and of the Deposit Formed in a
Heat Exchanger When the Skim Milk is
Heated to 85°C[a]

	Percentage of dry matter in	
Component	Skim milk	Deposit
Serum protein	6	34
Casein	31	11
Total salts	8	45
Ca	1.3	16
PO_4, inorganic	2.0	23
Sugars	53	0.0
Other	2	10

[a] Approximate example, after results by T.J.M. Jeurnink, P. Walstra, and C.G. de Kruif, *Neth. Milk Dairy J* **50** (1996) 407–426.

stone or scale. It is thus obvious that two milk components are preferentially deposited, i.e., serum protein and calcium phosphate. These are indeed the substances that become insoluble and supersaturated, respectively, at high temperature. This follows from Figure 6.9 for serum proteins and Figure 2.8 for inorganic phosphate, which show that below 70°C the changes occurring with these substances are very slow; this agrees with the observation that below 70°C little fouling occurs. It is thus clear as to which substances are primarily deposited and why, but the mechanism needs elaboration. Three mechanisms can be envisaged.

(1) *Temperature gradient near the wall*. When a vessel with liquid is heated from the outside, its inner surface will have a higher temperature than the liquid. If a reaction leading to insolubility proceeds faster at a higher temperature, some of the material may be deposited on the wall and its concentration is thereby lowered close to the wall. This implies that a concentration gradient is formed, inducing further transport of material to the wall, where part of it is deposited. This occurs in the heating section of a heat exchanger, where fresh liquid continuously flows past the wall. A temperature gradient, and thereby a concentration gradient, will only exist in a laminar boundary layer near the wall because any turbulence farther away causes intensive mixing (see Fig. 12.2). Nevertheless, this situation implies a continuous deposition, the more so as the temperature gradient is greater (larger ΔT between wall and liquid) and as the turbulence in the liquid is less intensive because that makes a thicker laminar boundary layer.
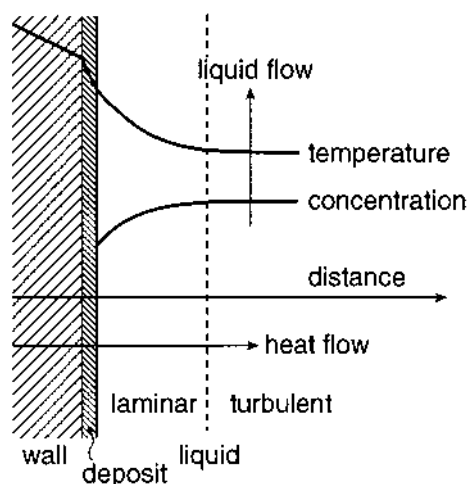
FIGURE 12.2   Temperature gradient near a heated wall in a flow-through heat exchanger and its effect on the concentration gradient of a material that becomes insoluble at high temperature and is thereby deposited onto the wall. Highly schematic.

The importance of this mechanism in milk heat exchangers can be evaluated from the effect of ΔT on fouling. It turns out that for the same milk temperature, fouling is stronger in the heating section ($\Delta T > 0$) than in the holding ($\Delta T = 0$) or cooling sections ($\Delta T < 0$), but not greatly so. Consequently, a temperature gradient is not essential. On the other hand, a very large ΔT does enhance fouling, especially for concentrated milks.

(2)   *Competition between surfaces*. Insoluble material may also be deposited on the surface of any particles present in the liquid. In milk, casein micelles are the obvious candidate, and it is indeed known that the insoluble serum proteins formed upon heating, as well as calcium phosphate becoming supersaturated at high temperature, become associated with the micelles. Since casein micelles are very close to each other in milk (of the order of 0.1 μm), hence to the wall of the vessel, it is only material very close to the wall that can become deposited onto it. However, in a heat exchanger there is a continuous flow of liquid past the wall. The specific surface area A of the casein micelles can be calculated from the values given in Table 3.5. This yields:

$$A = 6 \; \varphi/d_{vs} \approx 6 \times 0.06/0.1 = 3.6 \; \mu m^{-1} = 3600 \; m^2/L$$

In a plate heat exchanger, 1 L of milk would be at any moment in contact with about 0.5 m^2 of heating surface. In first approximation, it follows that 0.5/3600,

corresponding to 0.014% of the material involved, would be deposited onto the metal surfaces. The proportion has been determined for β-lactoglobulin at 0.14%. This would imply that the metal surface would be 10 times as ''attractive'' for the protein to deposit on as the surface of the casein micelles. Although the proportion deposited is quite small, the continuous supply of fresh milk means that eventually a substantial layer of deposit can be formed. In a heat exchanger, about 1000 L of milk may pass any surface per hour; for the example just given, this then would lead to a deposition of

$$3.2 \times 0.0014 \times 1000/0.5 = 9 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$$

of β-lactoglobulin (assuming the milk to contain 0.32% of it).

Even if no particles are present in the liquid, as in whey, a similar competition may occur. In whey, denaturation of serum protein leads to the formation of aggregates. These then compete with the walls of the heat exchanger for deposition of further material that becomes insoluble. However, the deposition on the aggregates is less efficient than that on casein micelles, and whey gives a faster fouling rate, for proteins as well as calcium phosphate, than milk under the same conditions.

(3) *Air/vapor bubbles*. At a hot surface in contact with a liquid, air bubbles of about 1 mm may readily form if the liquid contains sufficient air for it to become supersaturated at the high temperature. The air in the bubbles is, of course, saturated with water vapor. If a bubble remains at the surface, it can considerably enhance fouling. This is illustrated in Figure 12.3 for casein mi-
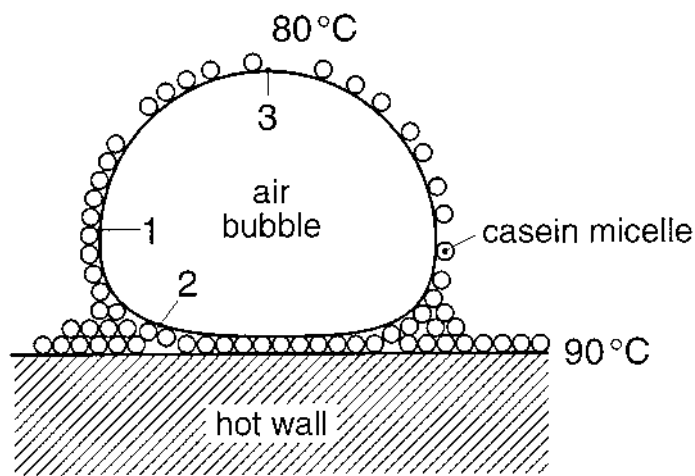
FIGURE 12.3 Air bubble formed at a heated surface and processes occurring near it. See text for explanation. Highly schematic and not to scale.

celles, but it applies equally well to other substances depositing, say, serum protein. Protein adsorbs onto the bubble (1). The bubble acts as an insulator, whereby the temperature at (2) is higher than at (3), say 90°C and 80°C, respectively. This causes water to evaporate near (2) into the bubble, and to condense near (3), whereby water and heat are transferred. Figure 9.6 gives that the relative water vapor pressures would be 0.72 and 0.48, respectively, and a substantial driving force for water transport thus exists. The liquid near (2) now becomes concentrated, leading to (greatly) enhanced deposition of protein. To overcome this problem, the milk should be evacuated before or during heat treatment, or the milk should be kept at a sufficiently high pressure during heating.

It appears that β-lactoglobulin plays a key role in most fouling of dairy liquids. As discussed in Section 6.2, it is transformed into another form at high temperature, where the -SH group buried in the interior becomes reactive, whereby it can induce formation of -S-S- crosslinks with other protein molecules. This reaction, as well as the formation of protein dimers and trimers, probably occurs in the bulk of the liquid, not at a surface. If, however, this aggregation reaction has been allowed to proceed almost completely, which means that termination of the polymerization reaction has occurred in whey, or that the serum proteins have become almost fully associated with the casein micelles in milk, further deposition would not occur. It is indeed observed that milk or whey that had been heated to such an extent that nearly all β-lactoglobulin has become insoluble (see Fig. 6.9) hardly shows any protein fouling.

Deposition of calcium phosphate just goes on, and the higher the temperature the faster. This explains the formation of milkstone at high temperature, where the serum proteins have already been denatured. When milk is heated to UHT temperatures in a heat exchanger, the part up to 70°C may show very little fouling, between 70°C and 100°C a type A deposit, and at higher temperatures, including the first part of the cooling section, type B.

Casein micelles would show little tendency for depositing because of their repulsive hairy layer (see Section 3.2). They become to some extent entrapped in the deposit via the association with denatured serum protein. Some fat globules also become enclosed, especially if the milk has been homogenized.

In practice, the following are important factors affecting fouling of heat exchangers:

a. *Temperature*. This has been extensively discussed. Below 60°C hardly any deposit is formed.

b. *Preheating*. The formation of type A deposit is governed by the denaturation of β-lactoglobulin. Heating to such an extent that by far the most part is denatured will greatly diminish fouling. This is especially important for the concentrating of milk in an evaporator. It can also be useful for heat treatment of plain milk. Heating milk quickly to a temperature where β-lactoglobulin dena-

tures at a reasonable rate and then keeping the milk for a while in a vessel with a small ratio of surface area to volume leads to denaturation without much fouling.

   c.   *Temperature difference* between heating medium and liquid. Although this does not have an overriding effect in most cases, an excessive difference should certainly be avoided.

   d.   The *formation of gas bubbles* at the heated surface markedly accelerates deposit formation. Degassing of milk prior to heat treatment can therefore diminish fouling. Rapid streaming or agitation may dislodge bubbles from the surface.

   e.   *Acidity.* The lower the pH of the milk, the quicker a protein deposit forms. Milk or whey, turned sour by bacterial action, can cause a heat exchanger to become readily blocked. This is presumably caused by the lower solubility of denatured serum protein at lower pH (see Fig. 6.3A). Mineral deposition is less at lower pH.

   f.   *Concentrated milk* shows much quicker deposit formation than plain milk. In addition to a higher concentration of reactive components, the higher viscosity (hence, higher wall temperature) and lower pH play a role.

   g.   There is a large *variation among lots* of milk, but the explanation is not quite clear. Colostrum causes serious fouling during pasteurization due to its high serum protein content. Protein degradation caused by proteolytic enzymes from psychrotrophs can markedly enhance deposit formation.

   h.   *Cold storage* of milk reduces deposit formation during heat treatment. The explanation is not clear.

   A completely different type of deposit can occasionally be formed by bacteria. *Streptococcus thermophilus*, for instance, may grow on the surface of a heat exchanger for milk at temperatures between 40°C and 50°C. Only a small fraction of the bacteria can attach themselves to the wall, but as soon as one cell is present it can grow out, forming a so-called biofilm, i.e., a mixed layer of cells and milk components. Several bacteria can form similar biofilms under various conditions.

12.2   CLEANING

Cleaning is primarily aimed at a thorough removal of material causing growth of microorganisms and removal of formed deposit that impairs the efficiency of the machinery. A satisfactory cleaning begins with the design of the equipment (smooth surfaces, no ''dead ends'') and of the manufacturing processes.

   The following are essential operations in a cleaning process for dairy machinery:

   a.   *Prerinsing*. Vigorous prerinsing with water can remove some 80% to 90% of the material not absorbed onto the equipment. The consumed

prerinse water should not contain much milk, partly to limit any loss of milk. Because of this, most milk residues should be removed before prerinsing.

b. *Cleaning steps*. A much applied method is first cleaning with alkali, followed by an acid rinse. Alkali (e.g., 1% NaOH) swells the outer layer of the type A deposit. The time needed for the alkali to reach the wall by diffusion is too long for a thick deposit layer. For a layer of 1 cm thickness, it would take a few hours. Presumably, cracks are formed in the layer and applying a sufficiently intense turbulence then causes break-up of the deposit, thereby detaching it from the wall. Strong alkali may cause the opposite, since it makes the outer layer rubbery and the deposit hard to remove. After rinsing with water, nitric or phosphoric acid is introduced to remove the scale. If the acid rinse is omitted, the alkali treatment may even increase the amount of calcium salts deposited. The latter deposit would also include protein, so contaminating organisms would have an increased growth potential. Obviously, all scale should be removed.

Instead of the mentioned two-step cleaning, one-step cleaning by using compound detergents can be applied. These detergents include a calcium-chelating agent. The above-mentioned rinsings with water and acid then can be canceled. Cleaning with alkali and acid is always applied after serious fouling of equipment (e.g., evaporators), whereas compound detergents are generally used for less tenacious fouling, as applies for heat treatment of plain milk.

c. *Final rinsing* with water is meant to remove cleaning agents. After acid cleaning, the acid should exhaustively be washed away if disinfection with sodium hypochlorite is subsequently applied. Otherwise, the steel would become corroded and below pH 5 chlorine gas can be released.

Among the various cleaning systems, circulation cleaning (cleaning in place, CIP) is primarily applied. Automated CIP units are used, and several cleaning circuits are often connected to one such unit. Achieving a satisfactory separation of the consecutive liquids is essential to restrict consumption of water and loss of chemicals. Efficient separation is facilitated by applying conductivity measurements, sometimes combined with determinations of pH, temperature, and/or turbidity.

12.3 DISINFECTION

The common aim of disinfection is to kill the microorganisms present on surfaces and thereby prevent contamination of the product during manufacture and packing. A satisfactory disinfection does not necessarily kill all microorganisms pres-

ent but reduces their number to a level where any quality and health risk can reasonably be assumed absent. Disinfectants can only attack if they can reach the microorganisms. Because of this, microorganisms in biofilms, i.e., encapsulated in product remnants or deposits, have an increased survival probability, even if a strong disinfectant is used. The organisms then may proliferate to considerable numbers during the period between disinfection and the next processing run. Furthermore, the action of a disinfectant is often restricted because it becomes inactivated by organic compounds present. Clearly, good cleaning should precede any disinfection. Combined cleaning and disinfection can only be used if just loosely connected deposit is present and if prerinsing removes most of it.

Most microorganisms are removed during cleaning. Moreover, some cleaning agents such as strong alkali and nitric acid solutions have a disinfecting action. Accordingly, separate disinfection is only required if too many microorganisms have been left after the cleaning. Aseptic filling machines should be disinfected just before manufacture starts, rather than after cleaning. This will not be necessary in other cases if cleaning is satisfactory.

Heat or chemical agents can be used for disinfection. In the former method, hot water or steam is used. Maintaining the required minimum temperature in the machinery and at the surfaces for sufficient time is essential. High temperatures cause denaturation of remaining proteins, and these can then precipitate on the equipment. Prior to heat disinfection, good cleaning thus is necessary. Disinfection by heat, especially with steam, has the additional benefit of enhancing the subsequent drainage and drying of the machinery, hence of diminishing the risk of bacterial growth. Moreover, after heat disinfection no disinfectant residues are left.

In the dairy industry, an aqueous solution of sodium hypochlorite, i.e., NaOCl, is the most important disinfectant used. Sodium hypochlorite is prepared by injecting chlorine into an NaOH solution. The following reaction occurs:

$$2NaOH + Cl_2 \rightarrow NaOCl + NaCl + H_2O \tag{12.1}$$

and in aqueous solutions:

$$NaOCl + H_2O \rightarrow NaOH + HOCl \tag{12.2}$$

$$HOCl \rightleftarrows H^+ + OCl^- \tag{12.3}$$
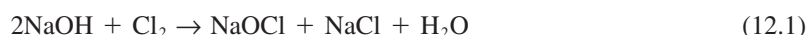
The undissociated HOCl is bactericidal. The degree of dissociation of Reaction (12.3) is smaller for a lower pH and the bactericidal effect is maximal at about pH 5. However, at pH 5 hypochloric acid is unstable and very corrosive. Because of this, Na hypochlorite is stabilized by the addition of a small excess of NaOH to achieve a pH of 8–9. During application, the dilution with water lowers the pH to a value at which the bactericidal agent is fairly active. During the final

rinsing step with water it is advisable that any acid left after the preceding cleaning step be removed because chlorine gas is formed in an acid atmosphere. Chlorine gas is corrosive and poisonous if inhaled. Instead of NaOCl, iodophors, hydrogen peroxide, and peracetic acid are also used as disinfectants.

Residues of cleaning agents and disinfectants should not be allowed to contaminate the finished products; thus, the final rinsing step is essential. Some agents, such as quaternary ammonium compounds, can absorb onto surfaces of equipment and such residues can eventually be taken up by the product.

SUGGESTED LITERATURE

- An interpretive review on fouling and cleaning is:
  T. J. M. Jeurnink, P. Walstra, and C. G. de Kruif, Mechanisms of fouling in dairy processing, *Neth. Milk Dairy J. 50*, 1996, 407–426.

13

Packing

Packing is an essential process step for most foods. The following are the main objectives.

a. Protection of the product against outside influences. This implies prevention of contamination by microorganisms, undesirable material (including oxygen), and dirt particles; prevention of loss of compounds (evaporation, aroma loss); exclusion of light and sometimes heat.
b. Partitioning of the product into units that are easy to handle during storage, transport, and consumption. Among the variables involved are portion size, shape, strength of the packing material, its resistance to temperature fluctuations, and the manner of opening the package and closing it again.
c. Guaranteeing a certain amount of product.
d. Transfer of information about the product and its origin. This may be kind of product and composition (nutritive value), keeping quality (e.g., date before which it should be consumed), advice on storage and use, name of manufacturer, various kinds of advertising matter and messages intended to strengthen customer relations.

Some aspects, mainly related to objective 1, will be briefly discussed. Liquid milk products in particular will be considered.

There are several *packing systems*. Moreover, milk and milk products may be sold unpacked. The product then is kept in a relatively large vat, and a certain amount of it is poured from this vat into a smaller vat of the consumer. The method is cheap with respect to materials but is labor-intensive. More impor-

tantly, contamination by microorganisms is inevitable. The contaminated milk will rapidly spoil and may contain pathogens, and it is thus highly advisable that the user boil the milk and clean the vat.

Packing in glass bottles (currently also in polyethylene or polycarbonate bottles) has the advantage that the bottles can be used many times, but the drawback that the return of the bottles, especially their cleaning and subsequent inspection, is laborious and expensive. The disadvantage of the great weight of the bottles may be acceptable in the case of home delivery.

Most milk is distributed in single-service containers. Containers for durable milk products are often made of tinplate or of various synthetic materials. For less durable products, plastics or laminates of cardboard and plastic are often shaped into cartons, sachets, or small cups. The contents may range from about 10 ml (coffee cream) to 3785 ml (beverage milk in some countries).

Another important variable is whether the packed product is sufficiently stabilized or still has to be processed (e.g., cooling, sterilization, shaking) or transformed (e.g., lactic acid fermentation, often with CO_2 formation). In-bottle or in-can sterilization implies heating under pressure in a moist atmosphere, and is predominantly applied to products packed in glass or plastic bottles or in cans; temperature control and closure of the package have to comply with the strictest requirements.

Still another variable is the stage at which the package is made. Compare the use of a previously prepared package that needs only to be closed after filling (glass bottle, can, some cartons and plastic bottles) to that of a package that is made and filled simultaneously (formation of cartons, blowing of bottles from extruded plastic, pressing of plastic cups from a foil). Sometimes (Tetra) a vertical cylinder is formed from laminated packing material (cardboard and plastic). It is supplied with milk while it is rapidly pulled down. The filled, moving tube is sealed and cut at regular distances so that tetrahedral or brick-shaped packs are formed. During filling, particular measures may be taken to prevent microbial contamination (aseptic packing).

The manufacturer's selection of a particular packing system depends on the specific requirements for the package, the extent to which the process can be fitted into the whole operation, the reliability, and the costs involved. Among other important aspects are environmental pollution and restriction of the use of nonreturnable package.

Several widely varying *materials for packing* are in use. The extent to which the materials meet various requirements and preferences will be briefly discussed. A number of characteristics are listed in Table 13.1. The data involved are highly approximative since they can vary widely according to the precise composition and way of manufacture. The list is far from exhaustive.

Processability. Is the material brittle, pliable, or mouldable; is it available in the desirable thickness (e.g., cellophane can only be made thin-walled); is it

TABLE 13.1   Properties of Some Packing Materials

Material	Strength	Flexibility	Sealability	Resists sterilization	Resists freezing	Translucency	Permeability to			
							H_2O 10^{-12} kg $\cdot$ m^{-1} $\cdot$ s^{-1}	O_2 10^{-18} kg $\cdot$ m^{-1} $\cdot$ s^{-1} $\cdot$ Pa^{-1}	CO_2	Fat
Glass	Brittle	0	—	Yes	No	Clear	0	0	0	0
Tinplate	Great	Small	—	Yes	Yes	0	0	0	0	0
Aluminum foil	+++	++	Not	Yes	Yes	0	<0.1	0.002	0.003	0
Paper/cardboard	++	++	Not	No	Yes/no	+	Great	Great	Great	Great
Cellophane	++	+++	Good	No	Yes	Clear	100	1	10	tr
Coated cellophane	++	+++	Good	No	Yes	Clear	1	0.1	0.1	tr
Polyethylene, L.D.[1]	+	+++	Good	No	Yes	+++	2	20	100	+++
Polyethylene, H.D.[1]		+++	Good	No	Yes	+++	1	5	25	++
Polyvinyl chloride	++	++	Fair	No	No	Clear	20	2	10	++
Polyamide (nylon)	++	++	Poor	Yes	Yes	Clear	40	0.3	1	tr
Polyester	+++	++	Poor	No	Yes	Clear	5	0.3	1	tr
Polypropylene	+++	Depends	Depends	Yes	No	Clear	2	10	50	++
Polystyrene	+++	+	Not	No	Yes	+++	10	20	100	++

Note: 10^{-12} kg $\cdot$ m^{-1} $\cdot$ s^{-1} corresponds to 3.5 g $\cdot$ m^{-2} $\cdot$ day^{-1} at a layer thickness of 25 μm; 10^{-18} kg $\cdot$ m^{-1} $\cdot$ s^{-1} $\cdot$ Pa^{-1} corresponds to 0.35 g $\cdot$ m^{-2} $\cdot$ day^{-1} at a pressure difference of 1 bar and a layer thickness of 25 μm.
[1] L.D., low density; H.D., high density.

fit to seal (especially by heat sealing), suitable for lamination (adhesiveness); can it readily be cleaned and sterilized; is it resistant to high temperatures, e.g., during in-bottle sterilization?

Resistance. Does the material resist damage? In other words, is it strong enough (this depends very much on its thickness) and wear-resistant? Can it withstand fluctuations in pressure and temperature, e.g., during sterilization, freezing (some plastics become brittle at low temperature), or gas formation? Is it resistant to a moist atmosphere, i.e., does it not soften? Does it not show rapid ageing? Some plastics rapidly become weak or brittle when exposed to light.

Permeability. Bacteria are generally not let through, provided that the closure of the package is perfect. Passage of a substance may be by diffusion through the packing material and, consequently, greatly depends on the solubility of the substance in the material. The amount of substance let through generally is proportional to contact area, time, and concentration difference (for gases often expressed as pressure difference), and inversely proportional to the thickness of the material. Consequently, the permeability can be expressed in, e.g., $kg \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$. Examples are given in Table 13.1. Considering transport of water, the loss of water into air of a certain relative humidity (often 85%) is usually taken as a basis. All permeabilities increase with temperature, for the most part as an Arrhenius relationship, in line with the temperature dependence of diffusion coefficients. These coefficients can greatly depend on the precise composition of the packing material. Compare, for instance, polyethylene of low and high densities. The latter is more compact due to its large proportion of unbranched chains. Plasticizers (softeners) mostly increase the diffusion coefficients considerably, and the plasticizer content can vary widely. Most plastics are hydrophobic, so the permeability to hydrophobic components (e.g., fat) is fairly large. Compare also CO_2 and O_2 in Table 13.1.

The above often does not apply if the layer becomes very thin (e.g., 25 μm or less) because the film can contain perforations. Aluminum foil is a good example because the permeability of aluminum to almost all substances is effectively zero, but any perforations cause trouble. Their number increases considerably with decreasing thickness of the foil and depends, moreover, on the production process and further handling in the dairy, during distribution, etc.

The permeability of the packing material naturally depends on its thickness. Often, containers composed of layers of different materials, so-called laminated foil, are applied. If the permeability to a certain component in a packing material of a given thickness is designated as b (expressed in, e.g., $kg \cdot m^{-2} \cdot s^{-1}$), the total permeability of a laminate can be calculated from $1/b_{total} = \Sigma(1/b_i)$.

Release of components to the food depends on the type of food (pH, presence of fat, etc.) and on the temperature. Plastics may release plasticizers, if still present, especially to high-fat products. Cans can release iron, tin, etc., and be-

cause of this, tin plate is always coated, i.e., supplied with a plastic layer. Uncovered cardboard may release several substances to the milk.

Heat insulation. Often a well-insulating package is not desirable, since after packing heating and/or cooling are to be applied. Although most plastics have poor heat conductivity, the layer often is too thin for satisfactory insulation. If insulation is needed, expanded polystyrene (polystyrene foam) can be applied.

Light transmission. For many foods a clear package is desirable, so that the user can see the contents. The drawback for milk products is that light-induced flavors (cardboard or sunlight flavor and oxidized or tallowy flavor) may develop. Cardboard is not translucent but is certainly not impermeable to light. Glass can be browned (above all, the short-wavelength light is harmful), but brown glass is often considered unattractive. Most plastics are quite translucent. Fillers can be applied to give color, and TiO_2 is often used to convey a white color.

Printability of the material often is important for the trade.

It will be clear that in many instances no single packing material meets all requirements. Because of this, laminates are applied. In a ''milk carton'' for durable, aseptically packed products we may find, going from outside to inside:

> Polyethylene: for water repellance.
> Paper: for printing purposes.
> Cardboard: for firmness.
> Polyethylene: to adhere cardboard to aluminum.
> Aluminum: against passage of light and compounds, including water.
> Polyethylene: for good sealability. Sealing here means closing the filled package by pressing while heating.

All of the layers are very thin (e.g., 20 μm, aluminum foil even thinner), except for the cardboard; a 1-L package weighs about 25 g, a glass bottle 400–600 g.

There are various *methods to fill a package* with a certain amount of liquid. Weighing is rarely applied. Bottles are usually filled to a certain level, but for highly viscous products a measuring pump should be used; one or a few turns by a plunger determines the amount of product delivered, nearly independently of the viscosity involved. Sometimes the filling step itself can cause problems because the high deformation rates applied may change the consistency of the product, which then becomes too thin. Accordingly, high-speed filling machines may be unsuitable for products like yogurt and custard.

The extent of *contamination by bacteria* during packing is essential for the keeping quality of the milk product. Relatively simple measures may yield substantial results, but strictly aseptic packing is far more difficult to achieve. For less durable products, contamination should be rigorously avoided if the product is heated before being packed. Accordingly, the packing material should be devoid of pathogenic microorganisms and contain few if any bacteria that can

grow during its storage. Satisfactory hygienic standards during production, transport, and storage of the packing material will prevent many problems because the materials involved are very poor substrates for microorganisms. Moreover, high temperatures and little water are used during manufacture of packing materials. Packages intended for repeated use (bottles) should be thoroughly cleaned before filling, and after cleaning they should be examined to remove dirty and damaged bottles. It is a known fact that consumers may put a milk bottle to other uses or provide it with barely removable objects. This involves a certain danger but produces no appreciable health hazard. After cleaning, the package is disinfected, e.g., with a sodium hypochlorite solution of 10 ppm activated chlorine if the milk product is not reheated. The bacterial count involved should not exceed 50 per bottle. If the product is heated after packing, the packing material causes few or no bacteriological problems. A major point is that leakage of the closure due to pressure differences occurring during cooling must be prevented.

In *aseptic packing* of durable products, spoilage of fewer than 1 in 10^4 packages—and preferably far less—may be considered acceptable. Pipes, storage tank, and surfaces of the packing machine come into contact with the sterilized product and have to be sterilized. The same holds for the packing material. Laminated paper has been shown to contain, say, 10 organisms per 100 cm^2, among which about 3% spores. The inner surface of a 1-L carton is about 800 cm^2 and will thus on average be contaminated by about 2.5 spores. These spores are the most heat-resistant, hence their number must be reduced to approximately 10^{-5}. Furthermore, the packages should aseptically be closed; an atmosphere with overpressure and sterile air is usually applied.

Sterilization of the packing material should not impair that material. Consequently, steam or hot water heating often is not possible. In most cases, sterilization with a hot (60–80°C) and concentrated (20% to 35%) solution of H_2O_2 is applied. Hot air (>100°C) can readily remove residues of H_2O_2, and it provides an additional sterilizing effect. H_2O_2 has an advantage over other liquid disinfectants in that it causes no serious problems with respect to residues left in the milk. Gaseous disinfectants such as ethylene oxide have a slow spore killing action and can only be applied if a long reaction time (several hours) is feasible. Since suitable light sources have been developed, sterilization by UV irradiation becomes increasingly prevalent, especially for packing materials and machines that are less readily sterilized by H_2O_2. UV light of 200–280 nm accounts for the sterilizing effect. If dust particles have become attached to the packing material, H_2O_2 will produce better effects due to its ''rinsing effect,'' whereas UV irradiation will be less effective due to particle shade. Clean-room techniques combined with irradiation is sometimes applied.

Aseptic packing has to be meticulously checked. Not only must the packed product be examined, but so must all preceding steps, as well as the operators, which are potential carriers of pathogens. If just one bacterium reaches the prod-

uct, and that bacterium is pathogenic and can proliferate (e.g., *Staphylococcus aureus*), the result could be disastrous. In addition to regular sampling during production, further samples will be taken at the times or situations known to go along with an increased risk of contamination. It is advisable to incubate these samples long enough, in most cases 5–7 days, at 30°C to allow sublethally damaged bacteria also to grow to detectable counts. The products should only be delivered if the result of the shelf life test is satisfactory.

SUGGESTED LITERATURE

*   A general overview of the packing of foods is given in Chapter 12 of:
        M. Karel, O. R. Fennema, and D. B. Lund, *Principles of Food Science*. II. *Physical Principles of Food Preservation*, Marcel Dekker, New York, 1975.
*   The packing of dairy products is described in:
        Technical Guide for the Packaging of Milk and Milk Products, IDF Document 143, 1982.

III

MILK PRODUCTS

14

Milk for Liquid Consumption

Liquid milk can be delivered to the consumer after various heat treatments: none (raw milk), pasteurized or sterilized; and either packed or not (although sterilized milk is, of course, always packed). The properties of liquid milk that require the most attention are safety to the consumer, shelf life, and flavor. Safety is, of course, essential and consumption of raw milk cannot be considered safe. Consequently, the delivery of raw milk is prohibited or severely curtailed in many countries. Likewise, delivering milk that is not packed may involve a health hazard.

The relative importance of other quality marks depends on usage. Milk can be consumed as a beverage, in which case flavor is paramount. Most consumers tend to dislike a cooked flavor, and therefore (low-intensity) pasteurization is to be preferred. Others use milk primarily in coffee or tea, in cooking, in baking, etc. Now the absence of a cooked flavor is mostly not essential (if not too intense) and shelf life may be the most important quality mark. Consequently, sterilized milk is often favored. One may even use milk preserves like evaporated milk, dried milk, or—for some uses—sweetened condensed milk.

Liquid milk may vary in composition. Often fat content is standardized to a value near that of average raw milk, but low-fat (semi-skim) and skim milks are also sold. Fortification with solids-not-fat or with protein is occasionally applied. Standardization to a specified protein content by means of ultrafiltration is another possibility, but it is generally illegal. Most countries have legal requirements for a minimum solids-not-fat or protein content.

14.1 PASTEURIZED MILK

Pasteurized beverage milk must be safe for the consumer and have a shelf life of several days when kept refrigerated. Flavor, nutritive value, and other properties should deviate only slightly from those of fresh raw milk.

The following contaminants can in principle be harmful to the consumer:

- Pathogenic microorganisms. These may already be in the milk while in the udder, or be incorporated during or after milking. Most of these do not survive pasteurization, but they may also enter the product by recontamination.
- Toxicants taken up by the cow (e.g., with the feed) and entering the milk during its synthesis.
- Antibiotics, used to treat (the udder of) the cow.
- Disinfectants used on the farm or in the plant.
- Bacterial toxins formed during keeping of the milk.
- Other toxicants entering the milk by contamination during and after milking.
- Radionuclides.

Pathogenic microorganisms can be killed by heating of the milk. Other contaminants can mostly not be nullified in this way. Obviously, proper cattle management, and an adequate way of collecting and handling the milk is necessary to prevent health hazards. Regular checks for the absence of contaminants are thus essential.

With reference to the shelf life and safety of the milk, most countries have legal requirements for the maximum allowed count of microorganisms (colony count), for the presence of coliforms, and for the absence of the enzyme alkaline phosphatase. To meet these requirements, the original milk should not contain too many heat-resistant bacteria (Chapter 4), the pasteurization step should be checked (recording thermometer and flow diversion valve), and contamination of pasteurized milk with microorganisms (or with raw milk) should be minimized.

A cream layer is less desirable, especially when nontransparent packing material is used. It can be prevented by homogenization, which implies that the pasteurizing intensity should be adapted to avoid lipolysis. The more intense the heat treatment, the more the flavor of the milk will differ from that of raw milk.

14.1.1 Manufacture

Figure 14.1 gives an example of the manufacture of pasteurized milk for liquid consumption.

The importance of *thermalization* to prevent fat and protein breakdown by heat-resistant enzymes of psychrotrophic bacteria is discussed in Chapter 6; see
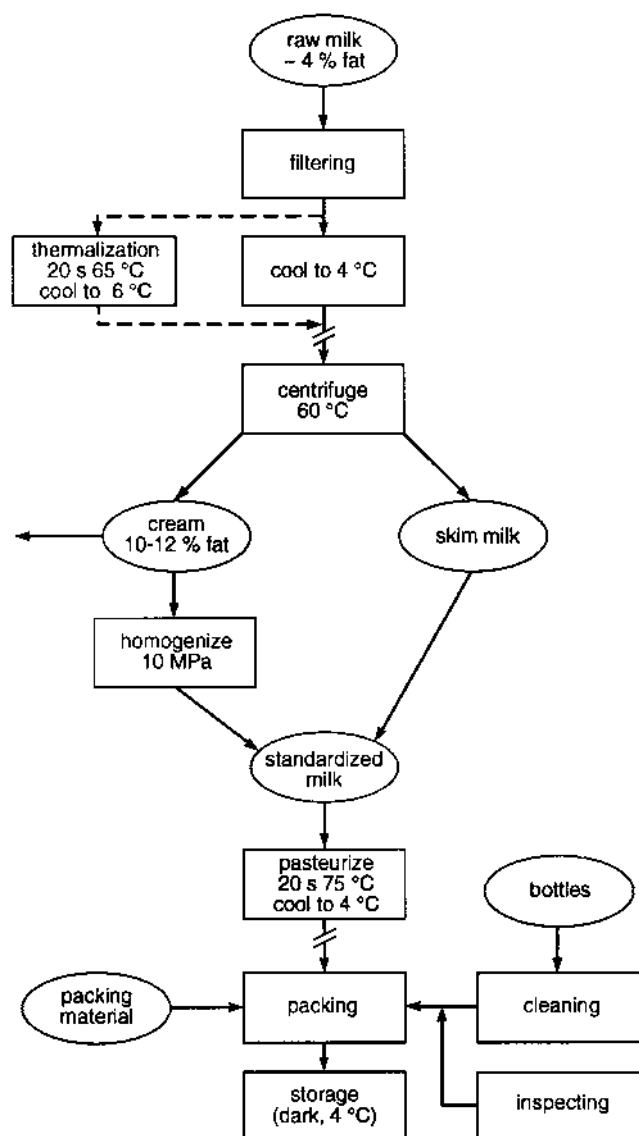
FIGURE 14.1 Example of the manufacture of homogenized, pasteurized beverage milk.

also Section 5.3. But as a rule, the keeping time of pasteurized milk is too short to cause noticeable decompositions by these enzymes, unless the original milk had a high count of psychrotrophs. Furthermore, thermalization at a rather high temperature (say 20 s at 67.5°C) causes a considerable inactivation of milk lipase (about 50%) and permits a somewhat lower pasteurization temperature in the manufacture of homogenized milk. Despite these obvious advantages of thermalization, dairy plants often only cool the milk (mainly because of the lower costs), taking the risk of some growth of psychrotrophs.

Separation is needed to adjust to the desired fat content. If homogenization is omitted, only a part of the milk will be skimmed, while the skim milk volume obtained should suffice to standardize the milk.

Homogenization serves to prevent the formation of a cream layer in the package during storage. Many users dislike such a layer. In low-pasteurized milk (alkaline phosphatase just inactivated) a loose cream layer of agglutinated fat globules forms that can be easily redispersed throughout the milk. In high-pasteurized milk the cold agglutinin has been inactivated and a cream layer forms far more slowly, but then it is a compact, hardly dispersible layer; a solid cream plug may even result from partial coalescence of the fat globules. Therefore, this milk is usually homogenized. As a rule, not all of the milk is homogenized but only its cream fraction (partial homogenization), to reduce cost. Obviously, all milk should then be separated. Homogenization clusters should be absent after the homogenization; therefore, the fat content of the cream should be rather low (10% to 12%) and the homogenizer temperature not too low ($\geq$55°C) (see Chapter 8). Usually the homogenization precedes the pasteurization, to minimize the risk of recontamination. Because milk lipase is still present, the milk should immediately be pasteurized (see Fig. 6.17).

After partial homogenization the milk may still cream due to cold agglutination. This results from the agglutinin being in the skim milk after warm separation and being not fully inactivated by the subsequent pasteurization (see Fig. 14.2). In spite of a low ratio of agglutinin to fat surface area, the fat globules can agglutinate if the raw milk contained much agglutinin.

Homogenized milk has an increased tendency to foam, especially at low temperature.

Standardization with respect to fat content is described in Section 5.4. It can be done by adding skim milk (or cream) to the milk in the storage tank or by continuous standardization.

Pasteurization ensures the safety and greatly enhances the shelf life of the product. A mild heat treatment, e.g., 15 s at 72°C, kills all pathogens that may be present (especially *Mycobacterium tuberculosis, Salmonella* spp., enteropathogenic *Coli* spp., *Campylobacter jejuni*, and *Listeria monocytogenes*), to such an extent that no health hazard is left. Some cells of some strains of *Staphylococcus aureus* can survive the heat treatment, but they do not grow to the extent as

to form hazardous amounts of toxins (see Table 6.4). Such low pasteurization inactivates alkaline phosphatase to the extent as to be no longer detectable (the enzyme may, however, regenerate after keeping the product for some days, but this especially holds for pasteurized cream). Most of the spoilage microorganisms in raw milk, such as coliforms, mesophilic lactic acid bacteria, and psychrotrophs, are also killed by low pasteurization. Among those not killed are heat-resistant micrococci (*Microbacterium* spp.), some thermophilic streptococci, and bacterial spores. But these microorganisms do not grow too quickly in milk, except *Bacillus cereus*. The latter organism is pathogenic if present in large numbers, but prior to this the milk is undrinkable because of its flavor.

After low pasteurization enzymic decompositions lipolysis (as caused by the natural milk lipoprotein lipase) is of special importance. Figure 14.2 shows the time–temperature relationships that reduce the activity of the enzyme to 10^{-1}, 10^{-2}, and 10^{-4}, respectively. Homogenized milk is highly susceptible to lipolysis because of its readily accessible ''substrate''; hence it should be rather intensely heated (e.g., 20 s at 75°C) to reduce its lipase activity to 10^{-3} or 10^{-4}. A decrease to 10^{-2} suffices for nonhomogenized milk, which implies a heating of, say, 15 s at 72.5°C. Milk proteinase is not inactivated by pasteurization (see Fig. 6.9); but the keeping time of pasteurized beverage milk generally is too short to cause problems.

After low pasteurization of the milk (15 s at 72°C), sufficient natural substances inhibiting bacterial growth remain intact, but a somewhat higher pasteurization temperature, as is needed for homogenized milk, clearly decreases their effect (see Fig. 14.2). It mainly concerns the immunoglobulins; their inactivation runs parallel to that of the agglutinins that determine the creaming properties. The agglutinins against bacteria (e.g., inhibitors of *B. cereus*) are also inactivated by homogenization and thus are absent in homogenized milk; they may remain active upon partial homogenization. The influence of the inactivation of bacterial growth inhibitors on the keeping quality of low-pasteurized beverage milk is illustrated in Figure 14.3 (see also Fig. 6.14). Heating for 15 s causes the decrease of the activity of the lactoperoxidase–thiocyanate–H_2O_2 system to become perceptible only at temperatures over 76°C. The effect of the presence or absence of inhibitors does, however, depend on the bacterial flora present. In high-pasteurized milk (e.g., 15 s 85°C) the bacterial growth inhibitors are eliminated and, despite its lower initial bacterial count, the milk may have a shorter shelf life than has low-pasteurized milk. High-pasteurized milk is often heated in the bottle, and this improves its keeping quality because recontamination does not occur; however, it also causes a distinct cooked flavor.

In the manufacture of low-pasteurized beverage milk flow-through heating is commonly applied, as a rule in a plate heat exchanger. The time–temperature combination selected is a compromise between sufficient inactivation of milk lipase and conservation of the activity inhibiting bacterial growth. Usually the
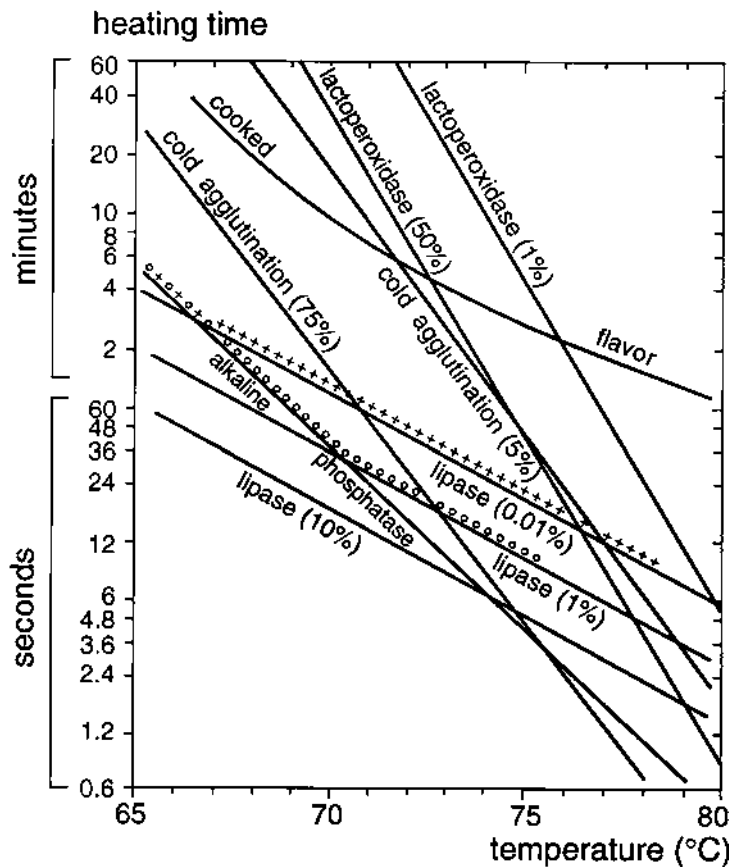
FIGURE 14.2 The heating time of milk needed to obtain certain effects as a function of temperature: inactivation of alkaline phosphatase to become ''nondetectable''; inactivation of lipoprotein lipase; inactivation of cold agglutination; inactivation of lactoperoxidase activity; and generation of a noticeable cooked flavor. The figures on the curves denote the approximate proportion of the activity left. Lower limits for low pasteurization of nonhomogenized (○○○○○) and of homogenized (+++++) milk are indicated.

temperature is adjusted, but as is seen in Figure 14.2, adjusting the length of time at a constant temperature may give better results (note that the slopes of the curves differ). On pasteurizing homogenized milk the agglutinins should be inactivated to such an extent as to prevent creaming of the milk. A cooked flavor may sometimes be observed.

colony count (ml^{-1})
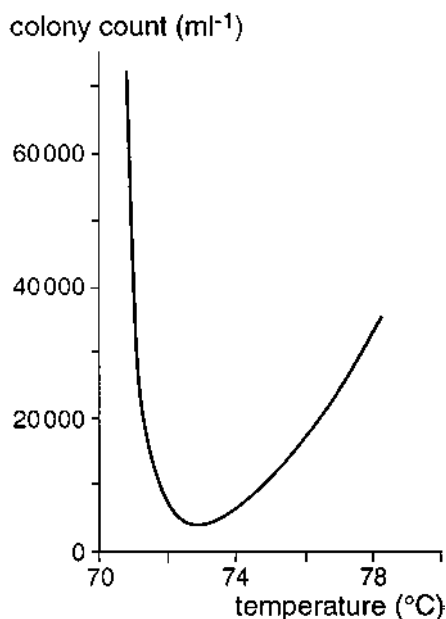
FIGURE 14.3 Example of the influence of the pasteurization temperature (pasteurization for about 20 s) on the bacterial count of unhomogenized milk after keeping it for 7 days at 7°C. Data kindly provided by M. P. Kimenai.

High-pasteurized milk has a somewhat whiter color [as has ultra-high-temperature short-time heated (UHT) milk; see Section 14.2], for the most part due to its homogenization. A more intense heating causes browning due to Maillard reactions. Sometimes heating to over 100°C is applied, to kill spores of *B. cereus*, thereby enhancing shelf life.

Packing of low-pasteurized beverage milk is generally done in single-service containers, such as cartons. A certain quantity of milk is still filled in glass bottles (see Chapter 13). Great care should be taken to ensure hygiene during packing in terms of the safety of the product, but especially because of the effect of recontamination on the shelf life of the product; aseptic packing is desirable. The temperature of the milk may increase by about 1 K during packing due to the transportation in pipelines and on conveyor belts, and due to the use of sealing machinery. Since recooling of packed products is slow, especially if piled up closely, such temperature increase should be anticipated by deeper cooling after pasteurization.

14.1.2 Shelf Life

Shelf life is the time during which the pasteurized product can be kept under certain conditions (e.g., at a given temperature) without apparent undesirable changes. Changes in beverage milk during storage can be distinguished in:

> Decompositions by bacteria growing in the milk, like acid production, protein breakdown, and fat hydrolysis
> Decompositions by milk enzymes or by extracellular bacterial enzymes, like fat and protein breakdown
> Chemical reactions causing oxidized or sunlight flavor
> Physicochemical changes like creaming, flocculation, and gel formation, which may in turn be caused by changes mentioned above

Changes caused by bacteria growing in the milk mostly do not become noticeable before their count amounts to $5–20 \times 10^6$ ml^{-1}, depending slightly on the bacterium species involved. If *B. cereus* is the spoilage organism, the limit taken is 10^6 ml^{-1}. Such counts should, however, not yet have been attained at the moment of purchase by the user. Pasteurized beverage milk should keep for several days after purchase, provided it is kept refrigerated (below 7°C). Sometimes, a ''day of ultimate sale'' is given with the product; in other cases an ''ultimate day of consumption'' (or minimum guaranteed shelf life). Criteria can be formulated for the bacteriological quality of the milk on the dates mentioned.

Enzymatic changes are described in Section 14.1.1. Chemical changes especially concern the high susceptibility of low-pasteurized milk to light-induced off-flavor.

Deterioration of pasteurized milk is especially caused by growth of microorganisms. It is determined by:

> Storage temperature
> Extent of recontamination
> Growth rate (generation time, *g*) of the bacteria involved
> Number of spores of *B. cereus* in the original milk
> Activity of substances inhibiting bacterial growth

The storage temperature of the milk is important because the generation time of the microorganisms is highly temperature-dependent, as is shown in Table 14.1 (see also Table 4.1). There is no real point in lowering the temperature to below 4–5°C because during transit and storage in the distribution network higher temperatures normally prevail, say 7°C. The effect of the temperature on the length of time that pasteurized milk can be kept is shown in Table 14.2.

The growth rate of bacteria depends on the temperature and the bacterium species involved. Starting from a count in the milk of 10 per liter, and with *g* amounting to 4, 7, and 10 h, a shelf life of 5, 8, and 13 days, respectively, is

TABLE 14.1 Generation Time (h) of Some
Bacterial Strains in Low-Pasteurized Milk
at Various Temperatures

Temp. (°C)	4	7	10	20
Bacillus cereus	∞	10	4	1
Bacillus circulans	20	12	10	3
Enterobacter cloacae	8	5	3	1
Pseudomonas putida	6	4	3	1
Listeria monocytogenes		20		
L. monocytogenes, in high-pasteurized milk	30	11	9	2

calculated. Such figures are quite normal. The shelf life of the milk at various temperatures may be predicted if the species of the bacteria involved as well as their initial count and generation time are known. Obviously, the shelf life of the milk depends on the growth possibilities of the bacteria present, whereas the total count just after pasteurization does not give sufficient information, as is illustrated in Figure 14.4.

After pasteurization of the milk its count usually amounts to 500–1000 ml^{-1}, unless many heat-resistant bacteria are present in the original milk. As a rule, the milk is spoiled by ''sweet curdling,'' caused by *B. cereus* ($g \geq 10$ h at 7°C), unless it is recontaminated (spoilage of recontaminated milk; see below). *B. cereus*, forming lecithinase, is also responsible for the ''bitty cream'' defect in nonhomogenized milk, i.e., the enzyme coagulates the fat globules in the cream

TABLE 14.2 Average Number of Days That Low-Pasteurized Milk
can be Stored at Various Temperatures Before it Reaches the Criteria
for the Guaranteed Day of Ultimate Sale (A) and of Guaranteed
Shelf Life (B), Respectively

Milk samples taken	Average number of days to obtain a count of					
	$5 \cdot 10^4$ ml^{-1} (A)			$5 \cdot 10^6$ ml^{-1} (B)		
	4°C	7°C	10°C	4°C	7°C	10°C
Just after the pasteurizer	>14	9.6	5.8	>14	13.6	9.8
From glass bottle	12.8	6.0	4.7	13.5	8.7	7.3
From carton	>14	7.8	5.2	>14	10.9	7.0

Data kindly provided by M.P. Kimenai.
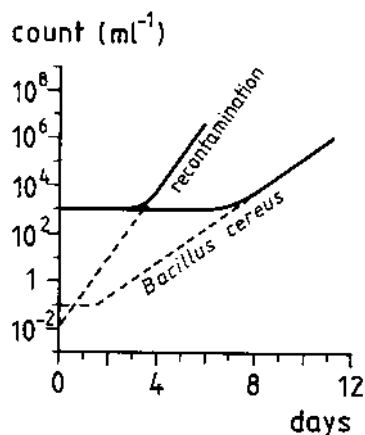
FIGURE 14.4 The bacterial count in low-pasteurized milk during storage at 7°C and the effect of recontamination. Approximate example. Solid line: total count; broken line: specific flora.

layer that are in the vicinity of a ''colony'' of these bacteria. At a storage temperature below 6°C, *B. cereus* cannot grow; deterioration may then be caused by *B. circulans*. High-pasteurized milk, made by heating at about 100°C, is mainly spoiled by *B. licheniformis*, or by *B. subtilis* if the keeping temperature is relatively high. Milk contains, say, 10 spores of *B. cereus* per 100 ml; its shelf life for normal storage conditions amounts to 12–14 days if it is not recontaminated. Shelf life can be extended by decreasing the count of *B. cereus* spores by means of bactofugation. Provisions should then be made against enzymic deterioration, while aseptic packing has to be applied.

If the pasteurized milk is *recontaminated*, deterioration is generally faster and of a different nature. This is illustrated in Table 14.2, in which the milk, leaving the pasteurizer, has not yet been recontaminated, but it commonly becomes so during packing. The presence of coliforms, detectable after keeping the milk at 20°C, is a good indication of recontamination having occurred. The (recontaminated) milk, stored uncooled, turns sour by the growth of, e.g., mesophilic lactic acid bacteria; high-pasteurized milk deteriorates quickest. Below 10°C the milk deteriorates by the growth of psychrotrophs ($g = 4$–5 h at 7°C). The flavor becomes putrid and rancid due to protein degradation and hydrolysis of fat, respectively. Since these psychrotrophs are hardly affected by substances inhibiting bacterial growth, the deterioration rate below 7°C is similar for both high- and low-pasteurized (recontaminated) milk.

The rule is that the more *B. cereus* spores in nonrecontaminated milk, or

the heavier the recontamination, the faster the deterioration. Thorough cleaning and disinfection of the filling and lidding machine is needed to avoid recontamination (as far as possible) after flow-through pasteurization. In determining the day of ultimate sale, one usually assumes that some recontamination of the milk occurs.

Frequent and thorough inspections are needed during processing to limit recontamination and to meet the requirements at the day of ultimate sale. To that end, samples may be kept at various temperatures and tested at intervals. The drawback is that the user has already received the milk before the result of the shelf life test is known. Tests have therefore been developed that allow a fairly rapid detection of recontamination by gram-negative, non-spore-forming bacteria.

14.1.3   Use of Microfiltration

By using microfiltration (Section 9.4), bacteria and bacterial spores can almost fully be removed from a solution; this can be advantageous in the manufacture of milk for liquid consumption. The method can readily be used in milk processing thanks to ceramic membranes and to further technological developments. Pressures applied are below 1 bar. A high flux and long operating periods can be achieved. The fat globules are also retained, considering that the membrane has a pore size of about 1 μm; therefore, the milk should first be separated. Figure 14.5 gives an outline of a manufacturing process. Some 0.1% to 1% of the total number of bacterial cells passes to the permeate, of *B. cereus* <0.05%. Stronger reductions, even up to sterility, can be obtained by using membranes with smaller pore size, but that is at the expense of the flux and of the maximum operating time. The amount of retentate is only a small percentage of the initial volume; the protein content is slightly increased, by about 0.5 percentage unit. The retentate is sterilized along with the cream.

The principal effect of the method is the enhanced shelf life of the product. On the other hand, part of the product (about 12%) is sterilized; the ensuing cooked flavor is restricted by applying a brief UHT treatment, but it is important to note that the fat globules (which generate the greater part of the sulfhydryl compounds on intense heat treatment) are in the most intensely heated fraction.

14.2   STERILIZED MILK

14.2.1   Description

Sterilization of milk is aimed at killing all microorganisms present, including bacterial spores, so that the packed product can be stored for a long period at ambient temperature, without spoilage by microorganisms. Since moulds and yeasts are readily killed, we are only concerned about bacteria. The undesirable
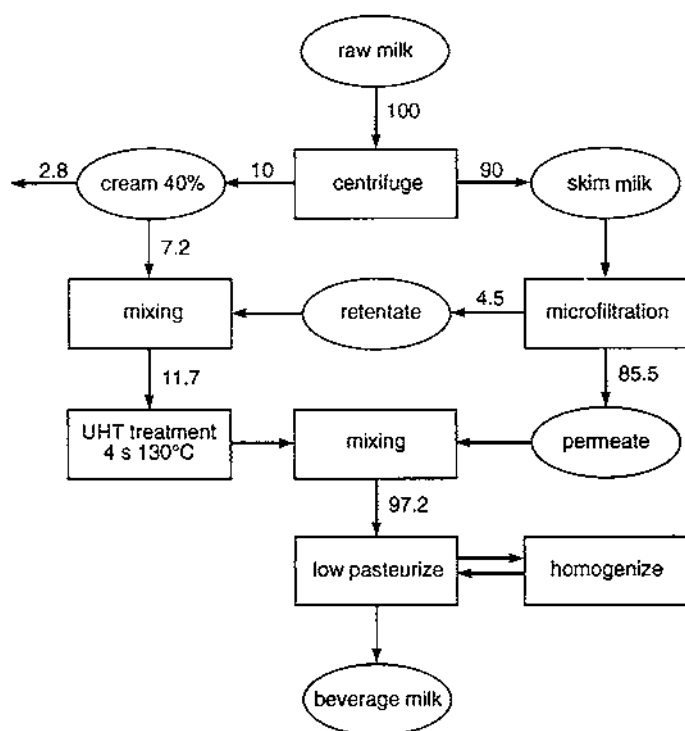
FIGURE 14.5   A manufacturing process for pasteurized beverage milk by using microfiltration. After P.J. Pedersen, IDF Special Issue 9201 (1992).

secondary effects of in-bottle sterilization like browning, sterilization flavor and losses of vitamins can be diminished by UHT sterilization. During packing of UHT-sterilized milk, contamination by bacteria has to be rigorously prevented. After UHT sterilization, certain enzymatic reactions and physicochemical changes still may occur.

To achieve the objectives it is necessary that:

The count of microorganisms, including spores, is reduced to less than 10^{-5} per liter.

The original milk does not contain enzymes of bacterial origin that cannot be fully inactivated by the heat treatment.

Enzymes naturally present in milk are sufficiently inactivated.

Chemical reactions during storage are minimal.

Physical properties of the milk change as little as possible during treatment and storage.

The flavor of the milk remains acceptable.

The nutritive value of the milk decreases only slightly.

These objectives and requirements are hard to reconcile. The most important ones determine what heating process will be selected. Furthermore, factors like processing costs, complexity of the machinery and processing, and, above all, the consumer's wishes must be taken into account.

The killing of microorganisms and the inactivation of enzymes are discussed in Section 6.3.

Oxidation causes off-flavors and decomposition of vitamins. Occurrence of these reactions during storage is limited by an intensive heating causing antioxidants to be formed, by deaeration, by excluding air from the package, and by using a package that is impermeable to light and oxygen. Furthermore, Maillard reactions can occur, both during the heat treatment (in-bottle sterilization) and during storage (UHT milk); see Section 6.2. The latter reactions are responsible for browning, off-flavor, and decreased nutritive value.

Sterilized milk is kept for a long time so that it will show strong gravity creaming if unhomogenized. Most intense creaming would occur in in-bottle sterilized milk because during the sterilization fat globules can coalesce. Creaming as such is undesirable. Besides, partial coalescence of the closely packed fat globules will lead to formation of a cream plug, which is hard to mix throughout the remaining milk; oiling off may even occur at somewhat elevated temperatures. Therefore, sterilized liquid milk is always homogenized.

If the milk is only in-bottle sterilized, little variation in process conditions is possible; the product obtained can be clearly recognized by the user because of its inevitable sterilized flavor. If the milk is UHT-heated, a sufficient sterilizing effect can readily be achieved, which implies that the appropriate process conditions can be selected on the basis of additional considerations. The flavor can vary from a mild (at, say, 0.6 s at 145°C) to a marked cooked flavor (UHT heating of, e.g., 16 s at 142°C in a heat exchanger with a warming and cooling profile, as shown in Figure 6.19, right-hand curve) that can be scarcely distinguished from the flavor of in-bottle sterilized milk. This makes it difficult to correctly characterize UHT-sterilized beverage milk and to clearly inform the consumer. Classification on the basis of the processing equipment involved is insufficient. Therefore, one has tried to characterize UHT milk by means of a chemical change, for which the formation of lactulose is generally used. A standard for UHT milk then would be that it contains less than 600 mg lactulose per liter.

14.2.2 Methods of Manufacture

Figures 14.6 and 14.7 give examples of the manufacture of sterilized milk. Thermalization, separation, and standardization are described in Section 14.1. The
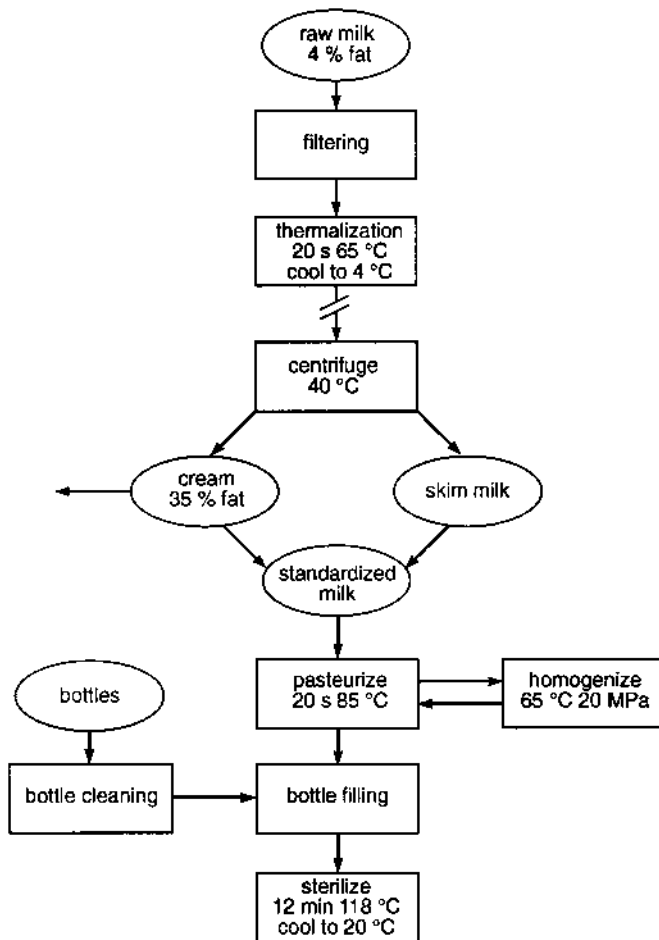
FIGURE 14.6 Example of the manufacture of in-bottle sterilized milk for consumption. Note: The pasteurization can be replaced by a UHT treatment with a considerable sterilizing effect, allowing a less intense in-bottle sterilization.

proteinases and lipases of psychrotrophs, especially of the genus *Pseudomonas*, can be very heat-resistant and even in-bottle sterilization does not suffice to fully inactivate these enzymes. Therefore, they should be absent in the raw milk. In particular, the addition of some milk left over for some time should be carefully avoided because in this milk psychrotrophs may have grown extensively. These bacteria especially produce heat-resistant enzymes in an (almost) full-grown culture (stationary phase).
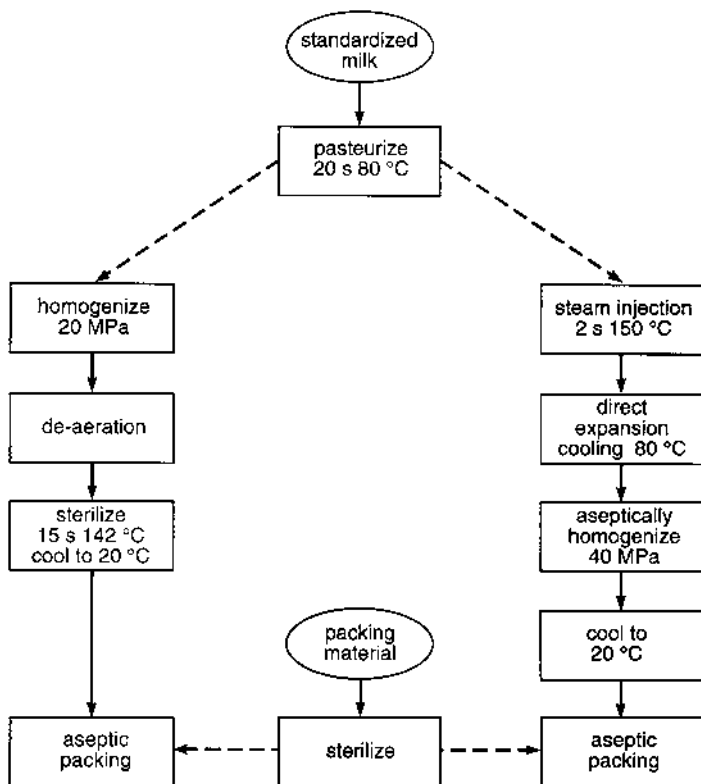
FIGURE 14.7 Examples of the manufacture of UHT-sterilized milk (indirect or direct heating) with aseptic packing.

The various types of heating processes are:

In-bottle sterilization
Flow-through preheating and a mild in-bottle sterilization
Flow-through sterilization and aseptic packing

The benefits and disadvantages of these types of heating processes, and the machinery involved, are discussed in Section 6.4. The sterilizing effect required determines the lower limit of the time–temperature relationship to be selected. The sterilization intensity also has an upper limit, which is reached when the milk protein starts to coagulate. Nearly all good-quality raw milk is stable enough to withstand sterilization (see also Section 6.2). The heating step in the UHT process with direct heating causes formation of aggregates of casein micelles,

which may lead to an astringent mouth-feel and to some sediment on storage
of the milk. Heat coagulation is responsible for the aggregates. High-pressure
homogenization (often 40 MP is needed) disrupts them; since homogenization
must be done aseptically, this needs a specifically designed homogenizer.

The temperature–time regime suitable for sterilization is depicted in Figure
14.8. Generally, a heat treatment above the line given for *Bacillus stearother-
mophilus* should be selected. Browning of in-bottle sterilized milk is inevitable
because at the usual temperature of 118°C the curves of sufficient sterilizing
effect and significant browning intersect.

UHT sterilization is mostly performed at temperatures above 140°C. Ac-
cordingly, the sterilizing effect required is readily attained. But a sufficiently long
shelf life at ambient temperature is only obtained if the residual activity of the
milk proteinase (plasmin) is at most 1%. (Many bacterial lipases and proteinases
are hardly inactivated during UHT heating and must thus be absent from the raw
milk.) Often, the curve for 600 mg lactulose represents the upper limit of UHT
sterilization, but at that limit a significant cooked flavor is already formed. Obvi-
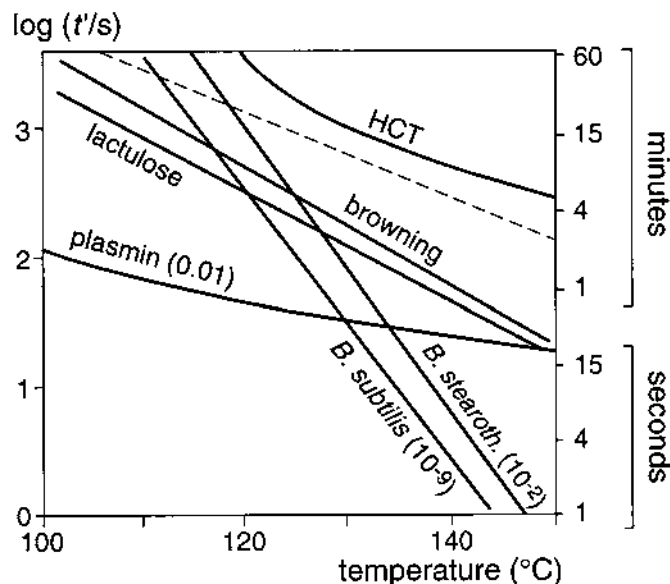
FIGURE 14.8 Changes in milk during sterilization: killing of bacterial spores,
inactivation of enzymes, and some undesirable changes such as a significant
browning; HCT = approximate heat coagulation time. The broken line very
roughly indicates a reduction of the activity of bacterial lipases and protein-
ases to 0.1. ''Lactulose'' corresponds to 600 mg/L.

ously, the suitable heating regime is restricted. For short sterilization times, however, both the selected time–temperature combination and the full ''thermal load'' of the product, including heating and re-cooling, are important (see Section 6.3).

When indirect UHT heating is applied, oxygen should first be removed from the product by means of deaeration, preferably up to less than 1 mg per kg milk. In direct heating this is already achieved during the evaporative cooling of the product. If a small amount of O_2 is present, it can lead to removal of a slight cooked flavor within a few days, but high O_2 contents cause development of an oxidized flavor and partial loss of some vitamins during keeping. Since the intense heat treatment during in-bottle sterilization forms sufficient antioxidants, deaeration is not necessary in that case (bottles with a crown cork become deaerated during sterilization).

The package for sterilized milk should be impermeable to O_2; on aseptic packing complete filling should be aimed at (no head space). UHT milk is, moreover, highly susceptible to off-flavors caused by light, so that a package impervious to light is to be preferred (see Chapter 13).

14.2.3 Shelf Life

Spoilage of in-bottle sterilized milk can be caused by insufficient heat treatment, due to which spores of, for instance, *Bacillus subtilis, B. circulans, B. coagulans*, or *B. stearothermophilus* have survived sterilization. *B. subtilis* has relatively heat-resistant spores and this bacterium may cause deterioration of in-bottle sterilized milk. If the milk is stored under tropical conditions, it may spoil due to *B. stearothermophilus*, which has very heat-resistant spores. Both a low count of these spores in the original milk and a UHT preheating step can help. *B. stearothermophilus* does not grow below about 35°C. A mild in-bottle sterilization after a UHT presterilization is only possible if during filling not more than a very slight contamination by bacterial spores occurs. If the package is not completely tight (e.g., due to an ill-fitting crown cork), then the milk can also be recontaminated and thus become spoiled. Enzymic or oxidative deterioration occurs hardly, if at all, because of the very intense heat treatment.

Deterioration of UHT milk by bacterial growth is usually caused by recontamination. Obviously, the type of deterioration is determined by the species of the recontaminating bacteria. Recontamination by pathogens may even occur, possibly without marked deterioration. Up to now some (rare) cases of food poisoning due to UHT milk contaminated by staphylococci have been reported.

Enzymatic deterioration of UHT milk due to the presence of heat-resistant bacterial enzymes, such as gelation or development of bitter, rancid, or putrid flavors, can only be prevented by a good-quality raw material. Deterioration by milk proteinase, causing, say, bitter flavor, will mainly occur in those cases where it is desirable to store UHT milk for a longer time (e.g., up to 6 months) and at

higher temperature, as in tropical countries. A more intense heating can partially prevent this. On the other hand, some dairies now produce a very briefly and directly heated UHT milk (e.g., 0.6 s at 145°C), the flavor deviating as little as possible from low-pasteurized milk, which it is meant to replace. Despite the absence of bacterial spoilage such milk will only keep for 2–3 weeks at ambient temperature because off-flavors like ''gluey'' and ''bitter'' develop, primarily due to plasmin activity. This period can be extended up to about 6 weeks if the product is kept refrigerated. Nonenzymatic deterioration of UHT milk during storage may concern: oxidation, influence of light, and Maillard reactions.

The keeping quality of in-bottle sterilized milk is checked by incubation of samples at various temperatures, mostly 30°C and 55°C. After a few days, one can, for instance, determine: smell, flavor, appearance, acidity, colony count, and oxygen pressure. The sterility of UHT milk can, in principle, be verified in much the same way. From a statistical viewpoint, a check of sterility of a large number of samples of any quantity is needed. Measurement of the O_2 pressure can be done rapidly, but it is only suitable if the product, just after packing, still contains some oxygen; reduction of O_2 pressure then points to microbial growth. Measurement of the increase in bacterial ATP via bioluminescence is also possible. The sterilized milk should preferably be sold only after the result of the shelf life test has become known and is satisfactory.

14.3 FLAVOR

Good flavor is, of course, an essential quality mark of beverage milk. Fresh milk has a fairly bland flavor, where full-cream milk has a ''richer'' taste than (partly) skimmed milk. The main aspect is, however, the absence of off-flavors. The fresh milk may already have off-flavors (see Section 2.6.4). These can mostly not be removed, although flavor compounds formed by heating may to some extent mask off-flavors; the first effect of heat treatment mostly is that the typical ''cowy'' flavor of fresh milk is reduced (or masked?), so that the flavor becomes even more bland. Flash boiling of milk, as occurs in the cooling section of a direct UHT heater or in a ''vacreator,'' may reduce some off-flavors.

Microbial growth, either before or after processing, may cause various off-flavors. Unclean or even putrid flavors are mainly caused by some psychrotrophs, whereas others (e.g., *Pseudomonas fragii*) cause fruity flavors. Lactic acid bacteria eventually cause the milk to turn sour, but other defects, such as a malty flavor (caused by *Lactococcus lactis* var. *maltigenes*), may also occur. *Bacillus circulans* occasionally causes a phenolic flavor in in-bottle sterilized milk. Growth of *B. cereus* in pasteurized milk readily leads to a very unclean flavor; this is fortunate because it prevents the consumer from drinking such milk, which might contain sufficient toxin to be hazardous.

Milk enzymes may cause a bitter flavor due to proteolysis by plasmin, as

may occur in UHT milk; and a soapy-rancid flavor, due to lipolysis by lipoprotein lipase in low-pasteurized milk. However, a soapy-rancid flavor is mostly due to lipolysis occurring prior to pasteurization or to action of heat-resistant microbial lipase originating from psychrotrophs. Lipolysis is discussed at length in Section 3.1.5.

Lipid oxidation may occur due to contamination of the milk with Cu or due to exposure to light. The resulting off-flavor is often called "tallowy" or just oxidized flavor, but in some cases a cardboard-like flavor develops. The latter may be caused by oxidation of phospholipids, and it can also develop in skim milk. The susceptibility of milk to obtain oxidized flavor is greatly variable, but it appears that rigorous exclusion of Cu (which implies a contamination of less than, say, 3 µg Cu per kg) and of light is always effective in preventing the defect to occur.

Exposure to light can be highly detrimental to milk flavor, and 10 min of direct sunlight on milk in a glass bottle or 10 h of "fluorescent" light on milk in a carton (that is not provided with an aluminum foil layer) may be sufficient to produce defects. The off-flavor is formed not immediately but rather several hours after illumination. It may concern oxidized flavors, but also a quite different "sunlight" flavor. The latter is mainly due to oxidation of free methionine to methional ($CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot CHO$) and to free thiols formed from sulfur-containing amino acid residues; the presence of riboflavin is needed for the sunlight flavor to develop.

Heat treatment leads to a change in flavor, the appreciation or dislike of which varies greatly among consumers. Every type of heat treatment causes its own flavor profile depending on the total thermal load of the process. The main flavor profile elements are cooked flavor, UHT ketone flavor, and sterilized-caramelized flavor. Cooked flavor is mainly caused by the presence of H_2S liberated after denaturation of protein (mainly from the fat globule membrane) during high pasteurization and boiling. UHT milk has also a cooked flavor but, in addition, it has a ketone flavor that predominantly originates in the lipid fraction and is due to methyl ketones and, to a lesser extent, to lactones and sulfur compounds. The cooked flavor as well as the ketone flavor greatly depend on the type of UHT process used. In-bottle sterilized milk has no real cooked flavor (-SH compounds); it has a UHT ketone flavor, but this is largely masked by the sterilized-caramelized flavor formed from certain Maillard and caramelization products. Often, the mild cooked flavor of UHT milk disappears partly during the first week after manufacture due to oxidation of the reducing sulfur compounds. Figure 14.9 gives an example of the flavor score of UHT milk, heated at various intensities.

14.4 NUTRITIVE VALUE

The nutritive value of pasteurized and UHT-sterilized milk changes little by the heat treatment and during storage. In-bottle sterilized milk shows a somewhat
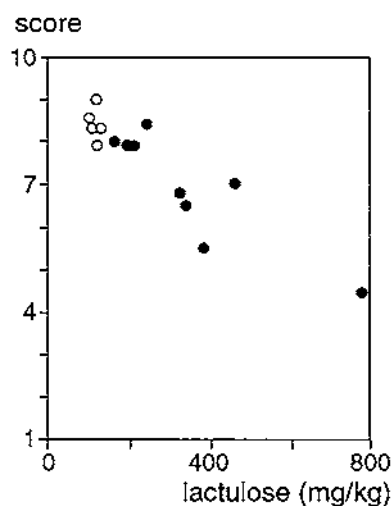
FIGURE 14.9 Average flavor score of UHT milk by a taste panel. Heating at various intensities is expressed by lactulose content. Direct (○) or indirect (●) heating. After P. Eberhard and P.U. Gallmann, Federal Dairy Research Institute, Liebefeld-Bern, Switzerland.

greater loss of nutritive value. Of special importance are the decrease of available lysine and the total or partial loss of some vitamins. Some data are given in Table 14.3.

Maillard reactions are responsible for the partial loss of lysine. They occur to some extent in UHT-sterilized milk during storage and in in-bottle sterilized milk during heating. The loss of lysine is not serious in itself because in milk protein lysine is in excess.

The losses of vitamins mainly concern vitamin C and some five vitamins of the B group. Vitamins A and E are sensitive to light and/or oxidation, but mostly their concentrations do not decrease in sterilized milk. Losses of vitamins in milk should be evaluated relative to the contribution of beverage milk to the supply of these vitamins in the total diet. Especially losses of vitamins B_1, B_2, and B_6 should be considered undesirable. The loss of vitamin C is generally of minor importance as such (milk is often not an important vitamin C source), but it may affect the nutritive value in other ways. The breakdown of vitamin C is connected with that of vitamin B_{12}; moreover, vitamin C protects folic acid from oxidation.

Loss of vitamins during storage can largely be avoided if O_2 is excluded (see Table 14.3). Vitamins C and B_9 may completely disappear within a few days

TABLE **14.3** Loss (in %) of Some Nutrients in Milk During the Various Heating Processes and Subsequent Storage (Very Approximate)

Treatment	Available lysine	Vit. B_1 (thiamin)	Vit. B_6 (pyridoxal)	Vit. B_9 (folic acid)	Vit. B_{12}	Vit. C
Pasteurization	0	5–10	0–5	3–5	3–10	5–20
UHT sterilization, directly	0	5–15	5–10	10–20	10–20	10–20
UHT sterilization, after 3 months storage	2	10–20[a,b]	20–50[a]	30–100[b]	20–50[b]	30–100[b]
In-bottle sterilization	5–10	20–40	10–20	20–50	30–80	30–60

[a] Dependent on exposure to light.
[b] Dependent on O_2 concentration.

if much O_2 is present. The loss is accelerated by exposure to light, with riboflavin (vitamin B_2) being a catalyst. Most of the riboflavin disappears on long-term exposure to light. The influence of the package on the permeability to oxygen and light is discussed in Chapter 13.

The nutritive value of fermented milks is discussed in Section 20.1.2.

SUGGESTED LITERATURE

- General information on beverage milk is in:
    Factors affecting the keeping quality of heat treated milk, IDF Bulletin, 1981, Document 130,
  and in:
    Monograph on Pasteurized milk, Bulletin of the International Dairy Federation No 200, 1986.
- Valuable information on UHT heating and aseptic packing is in:
    New Monograph on UHT Milk, IDF Bulletin, 1981, Document 133.
- Much about flavor and its sensory evaluation is discussed by:
    F. W. Bodyfelt, J. Tobias, and G. M. Trout, *The Sensory Evaluation of Dairy Products*, AVI, New York, 1988.
- Nutritive value of milk is discussed in:
    E. Renner, *Milk and Dairy Products in Human Nutrition,* Volkswirtschaftlicher Verlag, München, 1983.

15

Cream Products

Cream is sold in many varieties. The fat content may range from 10% (''half-and-half'') to 48% (''double cream''). Although it may be used for several purposes, mostly it is something of a luxury and therefore an excellent flavor is paramount. Because of the high fat content, any off-flavor of the fat becomes concentrated. For instance, a milk with a fat acidity of 1 mmol per 100 g fat will not be perceived to have a soapy-rancid flavor by most people, but a whipping cream made from it will definitely taste rancid. Hence, the milk should be impeccable with regard to lipolysis and fat oxidation.

Sometimes anhydrous milk fat is used in cream products and recombination is applied. This enhances the danger of an oxidation flavor and also, if impeccable in this respect, the taste of the product may be different (somewhat less rich) because of the absence of components from the milk fat globule membrane. One may improve on this by using a limited quantity of good-quality (dried) sweet cream buttermilk.

Besides plain cream, some derived products are made, such as sour cream (see Section 20.2) and ice cream. Here we will cover several products, chosen to illustrate most of the important technological and quality aspects of cream.

15.1 STERILIZED CREAM

This concerns cream with about 20% fat (light cream). A good keeping quality is essential because many consumers use it a little at a time or want to have it stored for special occasions. Accordingly, the cream is usually sterilized to guarantee microbial shelf life. Chemical stability is generally not a problem, although

ongoing Maillard reactions can occur during long-term storage. Due to the intense heat treatment oxidative deterioration scarcely occurs and neither does lipolysis. Physical deterioration may be considerable: gravity creaming and fat clumping or oiling off. Therefore, the cream must be homogenized. If stored for a long time it may thicken with age, form a gel, or become lumpy.

Most of the cream is used in coffee; hence the name coffee cream. Thus it is important that the cream not feather in coffee and that it cause sufficient whiteness (i.e., turbidity) after dilution with coffee. Likewise, no oil droplets should appear on the coffee. Sterilization flavor mostly is not too objectionable since this is largely masked in the coffee.

Cream is also used in desserts, e.g., on fruit. A pure flavor, then, is paramount, as are a white color and a relatively high viscosity. Sometimes a very thick, almost pudding-like cream is made.

15.1.1 Manufacture

Figure 15.1 gives an outline of the traditional manufacturing process for in-bottle sterilized coffee cream. Alternatively, raw or thermalized milk may be skimmed and the cream obtained may be standardized, pasteurized, and homogenized at the pasteurization temperature. A sterilizing effect of about 9 for *Bacillus subtilis* is mostly aimed at.

Figure 15.1 also shows the manufacture of ultra-high-temperature short-time heated (UHT) cream. In this case the cream should be homogenized after sterilizing; otherwise, UHT heating will cause coagulation of protein and fat globules, and coalescence of fat globules.

If a highly viscous cream is desired, the cream will be homogenized at a lower temperature and in one stage in order to produce a maximum of homogenization clusters.

15.1.2 Heat Stability

General aspects of heat stability are discussed in Section 6.2.

In making sterilized cream it is hard to avoid coagulation during sterilization while at the same time the product is sufficiently homogenized to prevent rapid creaming and (partial) coalescence of fat globules. Homogenization is largely responsible for the poor stability toward heat coagulation (see also Section 8.6 and Fig. 8.11). Although the heat stability of cream (like that of evaporated milk) can be improved by adjusting the pH and by adding stabilizing salts (e.g., citrate), the main variables are the conditions during the homogenization (see Fig. 15.2). It appears that as the surface area of fat globules that is covered with casein increases, the cream becomes less stable. Because of this, preheating at a high temperature does not help; it causes the serum proteins to precipitate so
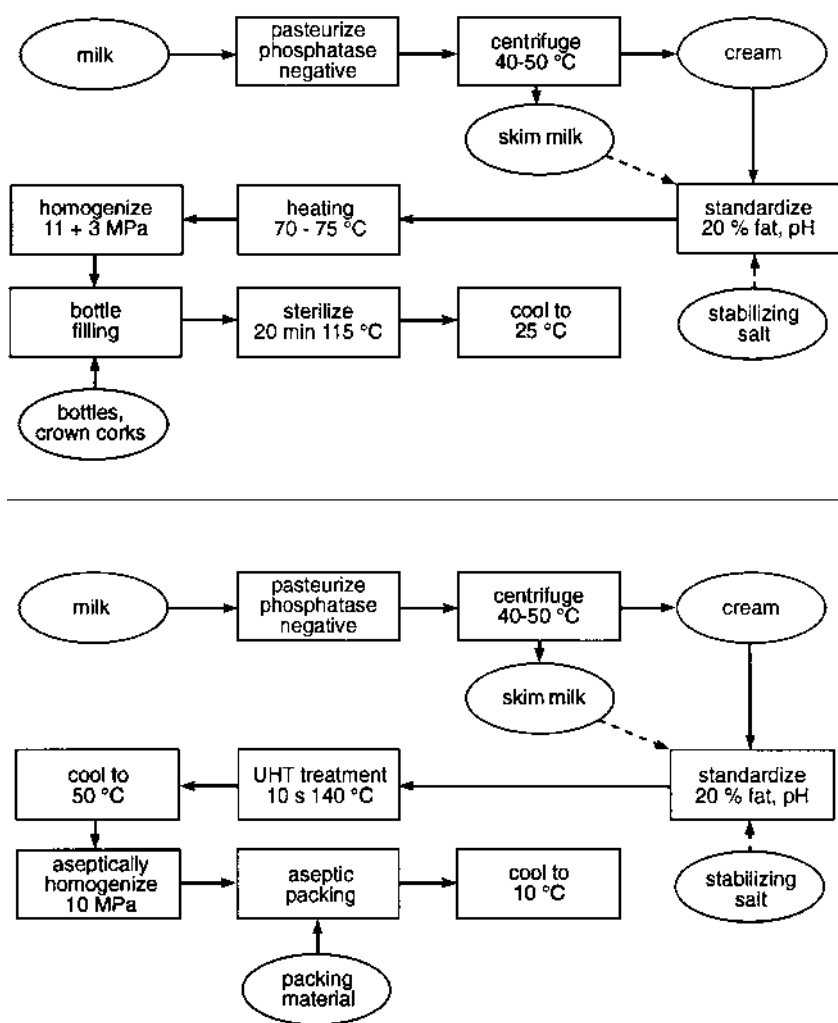
FIGURE 15.1 Examples of the manufacture of coffee cream (top) and dessert cream (bottom). Added stabilizing salt, e.g., is 0.15% $Na_3C_6H_5O_7 \cdot 5H_2O$.

that a larger part of the oil–water interface is covered by casein. Furthermore, the presence of homogenization clusters will shorten the heat coagulation time (Sections 8.7 and 15.1.4).

The higher the homogenization pressure, the lower the heat stability. How-
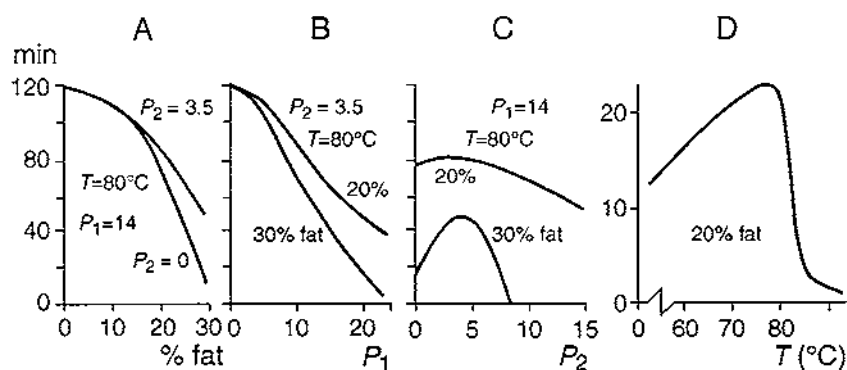
FIGURE 15.2 Heat stability (coagulation time at 120°C) of cream as related to the conditions during homogenization. P_1 is pressure before the first stage, P_2 before the second (MPa); T is homogenization temperature. A, B, C, tests in stationary cans; D, in rotating tubes. (From H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

ever, creaming and (partial) coalescence will cause problems at lower homogenization pressures. Hence, one must look for a compromise. It is advantageous to make the fat globule size distribution as narrow as possible.

15.1.3 Stability in Coffee

Feathering of the cream in coffee is due to coagulation of the fat globules and runs largely parallel to the heat stability. Consequently, UHT cream is rather susceptible to feathering. In its manufacture (Fig. 15.1) no problems do arise with heat coagulation, but feathering occurs readily if the homogenization pressure is too high. Moreover, UHT cream is liable to thicken with age (Section 16.1) or to show aggregation during storage. The latter phenomenon starts with the aggregation of fat globules. Soon this also leads to feathering in coffee. Feathering obviously depends on temperature, pH, and Ca^{2+} activity of the coffee, also. Stability in the coffee may be improved by increasing the solids-not-fat content of the cream, presumably as it buffers for H^+ and Ca^{2+} in the coffee.

15.1.4 Clustering

Dessert cream should be somewhat viscous. An obvious way is to achieve this by the formation of homogenization clusters, though thickening agents (carrageenan,
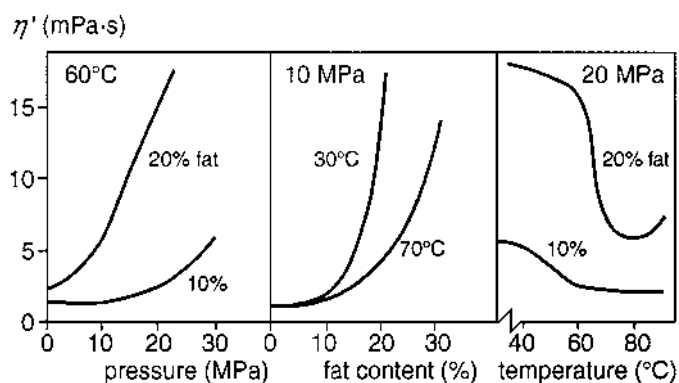
FIGURE 15.3 Influence of some process and product variables on the apparent viscosity (η') of homogenized cream. Approximate examples. Temperatures and pressures refer to conditions during homogenization. Measurements were at room temperature. (From H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

alginate) can be added successfully. Parameters affecting clustering are discussed in Section 8.7.

The main factors affecting the viscosity are summarized in Figure 15.3. At a given fat content the degree of clustering is responsible for the differences observed. Clustering increases the viscosity because the effective volume fraction of the fat globules increases: first, because of the plasma entrapped between the fat globules (this part of the plasma is essentially immobilized), and second, because of the irregular shape of the clusters (causing them to occupy an effectively enlarged volume when rotating due to the shear). As the fat content of the cream is higher, the increase in viscosity due to a given extent of clustering of the fat globules is stronger. Moreover, the clustering itself is more extensive for a higher fat content.

The viscosity can be reduced considerably by a second homogenization at a much lower pressure: the homogenization clusters then are partly disrupted again (hence reduced in size); moreover, the remaining clusters are more rounded. The same is achieved by exposing the ''clustered'' cream to shear, as, for instance, in a rotating viscometer. Figure 15.4 shows the ensuing decrease of the (apparent) viscosity with increasing shear rate; the greater the rate, the further the clusters are disrupted, and the latter do not reform on release of the shear, as the hysteresis loop shows. High shear rates should therefore be avoided during pumping and packing if homogenized cream is to retain its high viscosity.

Figure 15.4 shows that the viscosity decreases with increasing shear rate.
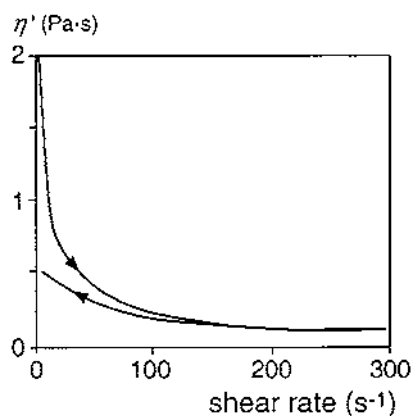
FIGURE 15.4 Example of the influence of the shear rate on the apparent viscosity (η') of homogenized cream (17% fat, homogenization at 40°C and 21 MPa); measurements at increasing shear rate were followed by those at decreasing rate. (From H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

In other words, the product is ''shear rate thinning'' and has an apparent viscosity. Figure 15.4 also shows that after the cream had been brought at high shear the viscosity remains low, even at a low shear rate. The shear rate should thus be specified and the cream be prevented from having first been exposed to higher shear rates, if the viscosity is to be a meaningful quantity. The consumer will usually apply very low shear rates, say some s^{-1}, when dessert cream is used (e.g., poured).

   If cream of a pudding-like consistency should be made it can be homogenized at such a low temperature that a small part of the fat is solid and true clumps of fat globules are formed. The product obtained is, however, very temperature-sensitive, so that warming to, say, 35°C causes loss of consistency and oiling off.

   Clustered cream hardly shows creaming after homogenization; this is because the whole contents of the bottle is like one big cluster. The latter may be compressed by gravity, causing a separated layer of milk plasma to appear at the bottom of the bottle.

15.2   WHIPPING CREAM

This concerns 35% to 40% fat cream. It is primarily designed to be beaten into a foam, often with sugar added. It is mostly available as a pasteurized product in small bottles, plastic cups, or large cans. It is also sold as in-can sterilized

cream, and even supplied with sugar and a driving gas in an aerosol can that delivers a ready-made whipped cream.

15.2.1 Desirable Properties

The most important specific requirements are:

1. *Flavor*. The product is eaten for its flavor, which obviously must be perfect. Rancid and tallowy flavors in the original milk should be rigorously avoided; this requirement is even more essential than for coffee cream. Not everybody appreciates a sterilization flavor or even a pronounced cooked flavor, and partly because of this the cream usually is pasteurized.

2. *Keeping quality*. Many kinds of spoilage can occur, but it is often desirable to store the cream for a prolonged time. The original milk should contain not more than a few heat-resistant bacteria; above all, *Bacillus cereus* is a disastrous microorganism in whipping cream (it causes the fat emulsion to become unstable). Nor should growth of psychrotrophs occur in the original milk because they form heat-resistant lipases. To allow for a fairly long shelf life, the pasteurized cream should be packed under strictly hygienic or even aseptic conditions. Recontamination by bacteria raises many complaints. Therefore, whipping cream is often heated by in-can or in-bottle pasteurization.

   Contamination by even minute amounts of copper causes autoxidation and hence off-flavor. Some coalescence of the fat globules during processing can readily lead to cream plug formation during storage. A cream plug implies that the product can hardly be removed from the bottle; moreover, it will readily churn rather than whip during beating in of air.

3. *Whippability*. The cream should quickly (i.e., in a few minutes) and easily whip up to form a firm and homogeneous product, containing about 50% v/v of air (=100% overrun).

4. *Stability after whipping*. The whipped cream should be firm enough to retain its shape, remain stable during deformation (as in ''decoration''), not exhibit coarsening of the air cells, and show negligible leakage of liquid.

Sometimes carrageenan is added as a thickening agent.

15.2.2 Manufacture

The classical manufacture of whipping cream is fairly simple; an example is shown in Figure 15.5. The pasteurization of the cream should at least be sufficient to fully inactivate milk lipase. Usually, the heat treatment is far more intense in
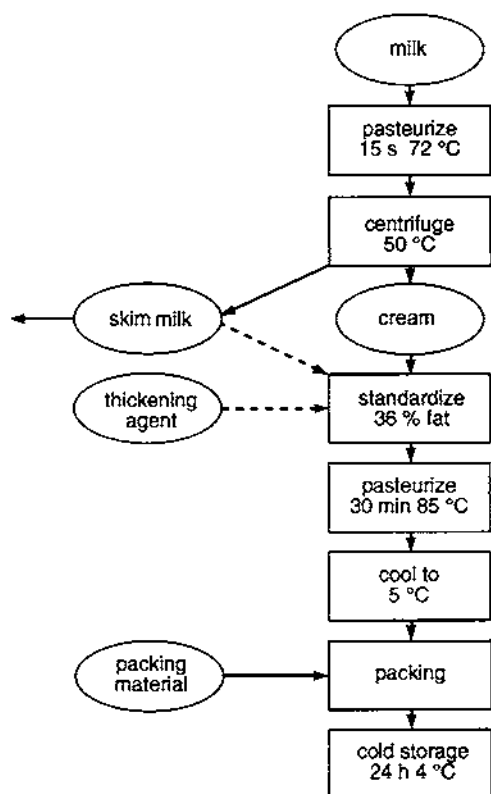
FIGURE 15.5 Example of the manufacture of whipping cream.

order to improve the bacterial keeping quality. The way of heating, as well as the heating intensity, varies widely; holder pasteurization (e.g., 30 min at 85°C), heating in a heat exchanger (possibly over 100°C), and in-can (bottle) heating (e.g., 20 min 103°C) are used. Likewise the manufacturing sequence, separation temperature, and so forth vary widely. Sometimes the cream is stirred in an open vat at rather high temperature in order to deodorize it; vacreation is not suited because it damages the fat globules.

Such damage, especially (partial) coalescence of the fat globules, should be avoided. The milk, and especially the cream, should be handled gently. The cream should not be processed or pumped unless the fat is completely liquid or largely solid, i.e., only at temperatures below 5°C or above 40°C. Hence, bottle filling of hot cream followed by cooling would be preferable, but it is rather uneconomical.

Sterilization of whipping cream may cause problems. In-bottle or in-can sterilization often causes coalescence, unless the cream is first homogenized. However, most homogenized cream cannot be whipped (Section 15.2.4). Accordingly, UHT heating is to be preferred, also because of the flavor (direct UHT heating causes strong homogenization); the cream should then be homogenized aseptically at low pressure and the composition should be adjusted (''emulsifier'' added). A disadvantage of UHT whipping cream is that the temperature fluctuations to which it may be subject (it is often stored uncooled for a time) can cause ''rebodying'' (Section 3.1). This implies a considerable increase in viscosity that, moreover, strongly impairs the whipping properties (churning rather than whipping).

To be readily whippable on delivery, the cream needs first to be kept refrigerated for a day in order to ensure that all fat globules contain some solid fat. To prevent creaming during storage, a thickening agent is generally added (e.g., 0.01% κ-carrageenan).

15.2.3   The Whipping Process

Interactions of fat globules with air bubbles are discussed in Section 3.1.

When skim milk is beaten, a foam very rich in air is rapidly formed on top of the liquid. This proceeds more slowly when cream is beaten and the air bubbles stay in the liquid for a longer time. This is partly because of the higher viscosity but also because the fat globules directly penetrate the air–water interface, attaching themselves to the air bubble and spreading some liquid fat onto the bubble surface. Because of this the films between closely approached air bubbles are rather unstable and initially the bubbles coalesce readily. The fat globules are so highly concentrated that they readily show partial coalescence (clumping). In this way a structure of clumped fat globules forms, enclosing the air bubbles and giving a rigid and stable foam. To achieve this, air cells and fat clumps should be of similar size, preferably 10–100 μm. The foam increases in firmness during whipping, but it also becomes coarser. On prolonged beating, the clumps become so large and few that they cannot stabilize but a few large air cells: the whipping becomes churning and the clumps become butter grains; the air bubbles coalesce and disappear again. These changes are illustrated in Figure 15.6.

The balance between foaming and churning partly depends on the way of beating. If this is too slow, the cream may churn prematurely. Vigorous beating causes a high overrun and a finely structured and smooth foam. The smaller the air cells, the less clumping is needed to enclose the bubbles and to produce a firm foam.

It is also possible to foam an emulsion without clumping occurring. Such a product may be sold in aerosol cans; thus it is not beaten, but the foam forms
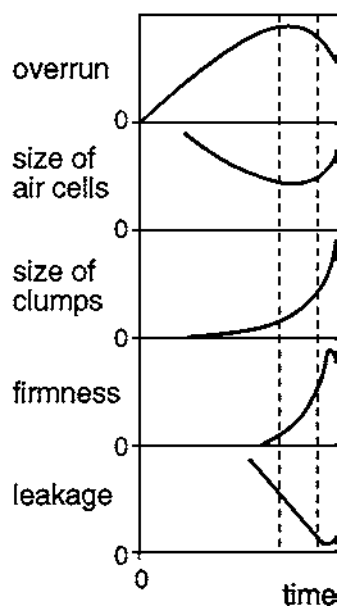
FIGURE 15.6 Processes occurring during whipping of cream. The parameter of firmness may be the time needed to lower a weight into the product; leakage means the amount of liquid drained from a certain volume in a certain time. Between the broken lines the product is acceptable. Very approximate. (After H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

when the gas pressure is released. Obviously, time does not suffice for sufficient clumping to occur. The fat globules curtail the overrun. They should not destabilize the air bubbles. This may be achieved by considerably reducing globule size. Proteins or other surfactants may cause some foam stability. But since encapsulation of air bubbles with fat globules does not occur, the foam is mostly unstable to manipulation and it soon becomes coarser due to Ostwald ripening of the air cells. On the other hand, these products often have a high overrun, over 200%, instead of around 100% for ordinary whipped cream.

15.2.4 Variables

Several properties of the cream affect the whipping process.

>   a. Fat content has a considerable effect (see Fig. 15.7). But the influence depends on the conditions during whipping. The more intensive the
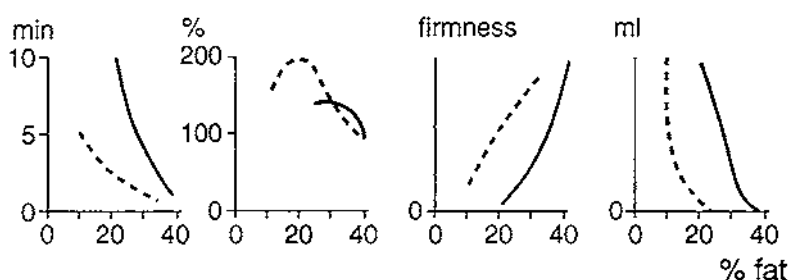
FIGURE 15.7 Properties of whipping cream. Whipping time (min), overrun (%), firmness (approximately a yield stress), and leakage of liquid (ml) as a function of the fat content, for conventional whipping cream (—) and for a product with emulsifier added (---). Very approximate examples. (From H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

beating, the lower the fat content of the cream allowing a stable foam to form, and the higher the overrun.

b. Crystallization of the fat is essential for clumping. If the amount of liquid fat is high, clumping is too rapid and the foam becomes unstable. Hence, deep cooling and a sufficient cooling time of the cream are essential, as is a low temperature during storage and at whipping. Obviously, the composition of the fat has an effect: There may be more problems in summer than in winter.

c. Further composition of the cream. Presumably protein is needed, especially when beating starts, to form foam cells. Addition of thickening agents hardly affects whipping, but leakage of liquid is considerably reduced.

d. Homogenization considerably impairs the whippability; the globules become too small to clump rapidly. This may, however, be better than expected if the fat globules have formed homogenization clusters because far less clumping is needed in that case. Homogenization at low pressure (1–4 MPa), preferably in two stages (e.g., 2 and 0.7 MPa at 35°C), can give clusters of some 15–20 μm in diameter.

e. Supplying the surface layers with other surface-active substances decreases the formation of clusters and increases the tendency to clumping; then homogenization at higher pressure may be applied. The surfactant added may be a monoglyceride or a Tween; the latter drastically affects the whipping properties (see Fig. 15.7).

15.3 ICE CREAM

There are numerous types of ''edible ice,'' essentially mixtures of water, sugar, flavor substances, and other components, which are partly frozen and beaten to form a rigid foam. In most types, milk or cream is an important ingredient. Some examples of the composition are given in Table 15.1. Nowadays, a part of the milk solids-not-fat is often substituted by whey constituents, to lower ingredient costs. In some countries, the fat is often substituted by vegetable fat, e.g., partly hydrogenated palm kernel oil. Dairy ice cream is the product discussed here.

Furthermore, soft serve, ordinary, and hardened ice cream are distinguished. Soft ice is eaten while fresh. It is made on the spot, its temperature is usually −3 to −5°C, and hence it still contains a fairly large amount of nonfrozen water; generally its fat content and overrun are rather low. Hardened ice cream, usually packed in small portions and sometimes supplied with an external chocolate coating, is much lower in temperature (e.g., −25°C), it hardly contains unfrozen water, and it is thus very hard; it has a long shelf life (several months). Ordinary ice cream has a lower temperature than soft ice cream (−10 to −15°C), but is not so cold as to be entirely solid; it is stored (a few weeks at most) in cans, from which portions can be ladled out.

Milk or cream of impeccable flavor is needed, especially with respect to rancidity and autoxidation. The latter defect may occur in hardened ice cream because it is stored for long times and its water activity is rather low; it contains a great deal of oxygen. Hence, contamination by copper has to be rigorously avoided.

Soft ice cream often causes microbiological problems, though it is kept cold and its high sugar content may, to some extent, act as a preservative. Pathogenic organisms will not grow, but nor are they killed. Bacteria are enabled to grow if the temperature becomes too high, locally and/or temporarily, which can easily

TABLE 15.1 Approximate Composition (Percentage by Weight) of Some Types of Ice Cream

Constituent	Dairy ice cream	Ice milk	Sherbet	Popsicle
Milk fat	10	4	2	0
Nonfat milk solids	11	12	4	0
Added sugar	14	13	22	22
Additives	0.4	0.6	0.4	0.2
% overrun[1]	100	85	50	∼0
Edible energy, kJ/100 ml	390	300	340	370

[1] % overrun means the relative increase in volume by air beaten in.

happen in practice in vending places. Abundant growth can occur in poorly cleaned processing equipment and in the mix, if stored for too long. Hence, strict hygienic measures have to be taken. Large numbers of Enterobacteriaceae (*E. coli, Salmonella* spp.) are frequently found.

15.3.1 Manufacture

Figure 15.8 gives a flow sheet in which cream is the starting material. Often one starts from skim milk powder and sweet cream butter or anhydrous milk fat. Other ingredients are whey powder or demineralized whey. In these cases recombination thus is needed.

The first stages of the manufacture need little elaboration. Composing the *mix* is relatively simple. The additives are emulsifier, stabilizer (a thickening agent, usually a mixture of polysaccharides), flavors and color substances. The role of the additives is discussed in Section 15.3.3. Clearly, ingredients such as fruit pulp and ground nuts should be added after the homogenization.

The *pasteurization* of the mix primarily serves to kill pathogenic and spoilage microorganisms. Additives added after the homogenization should usually be pasteurized separately. The second important objective is to inactivate lipase because it is still a little active even at very low temperature. Bacterial lipases should thus be prevented from occurring. Finally, quite intense heating of the mix is desirable (especially for hardened ice cream) to decrease its susceptibility to autoxidation; a cooked flavor may be undesirable, according to the added flavor substances.

The *homogenization* is specifically meant to give the ice cream a sufficiently fine smooth texture (see Section 15.3.2). Excessive formation of homogenization clusters should be avoided because it causes the mix to become highly viscous and the desirable fine texture to be not achieved; consequently, the homogenization pressure should be adapted to the fat content, to the pasteurization intensity, and, if need be, to the further composition of the mix (see Section 8.7).

The *cooling* and *ripening* (keeping cold for some time) are desirable for two reasons. The fat in most of the fat globules should largely be crystallized before the ice cream mix enters the freezer; it is important to note that considerable supercooling may occur because the fat globules are very small (Section 2.3). Furthermore, some components, especially certain stabilizers like gelatin and locust bean gum, need considerable time to swell after dispersing them.

The *freezing* implies rapid cooling of the mix to a few degrees below zero; in this way, ice is formed while air is beaten in. This must run simultaneously; after the bulk of the water is frozen, any beating in of air becomes impossible, and freezing after air is beaten in leads to insufficient ''churning'' of the fat globules (see below) and can damage the foam structure. Moreover, the vigorous
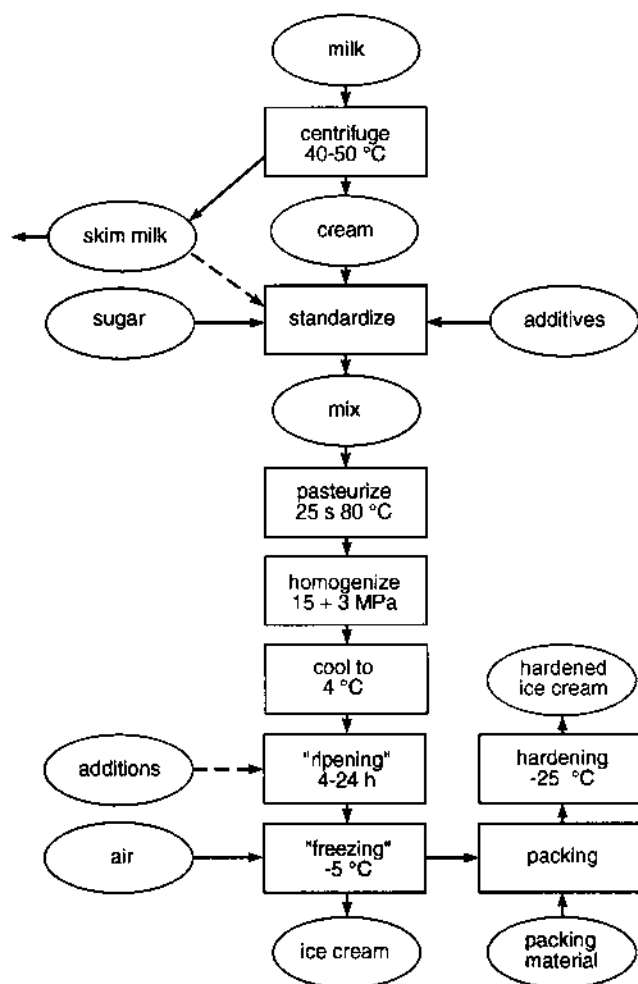
FIGURE 15.8 Example of the manufacture of ice cream.

beating enables rapid cooling, due to which small ice crystals can be formed. Figure 15.9 gives the approximate amount of frozen water as a function of temperature; a different composition causes a somewhat different curve. Usually, freezing is done in a scraped–surface heat exchanger—essentially a horizontal cylinder that is cooled externally by means of direct evaporation (-20 to $-30°C$) and equipped with a rotating stirrer (150–200 rpm) that scrapes the wall. A layer of ice is formed onto the wall. Pieces of ice are broken from the layer by the
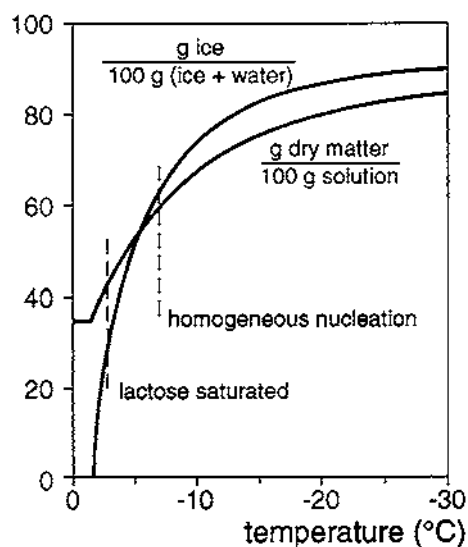
FIGURE 15.9 Freezing of ice cream mix. Approximate quantity of frozen water and concentration of the remaining solution, assuming that the ice is in equilibrium with the liquid and that no other constituents crystallize. The estimated temperatures for saturation of lactose and for its homogeneous nucleation are also indicated.

scraper and are distributed throughout the mass. A layer of ice about 50 μm thick is left. In its simplest design the cylinder is partly filled and the stirrer beats air cells into the mix. In continuous working machinery air and mix enter the equipment in predetermined volume quantities (allowing the overrun to be exactly adjusted), while the stirrer reduces the air cells in size. The process of manufacture takes a few minutes. The mix leaves the freezer at −3.5 to −7°C. A second heat exchanger may be applied, in which the mix is cooled further, while stirred, to about −10°C, without additional beating in of air. Deeper cooling cannot be achieved in a flow-type exchanger because the product becomes too firm.

The *packing* of ice cream often is a complicated operation, especially if mixtures or exceptional shapes are wanted. In the latter case the packing step may be associated with the start of the hardening in order to give the portions appropriate shape retention.

The *hardening* process serves to rapidly adjust the temperature of the ice cream to such a level as to retain its shape and to give it a sufficient shelf life with respect to chemical and enzymatic reactions as well as to the physical structure. The packed ice cream can be passed through a so-called hardening tunnel,

in which very cold air (e.g., $-40°C$) is blown past the small packets for some 20 min. Likewise, packed ice cream can be passed through a brine bath of low temperature.

15.3.2 Physical Structure: Formation and Stability

The chemical composition of an ice cream mix with air on top is exactly equal to that of the corresponding ice cream. All the same, the differences in appearance, consistency (mouth-feel), and flavor are considerable, and these are caused by the difference in physical structure. This is illustrated in Figure 15.10. When half of the water is frozen (about $-5°C$) the following structural elements can be distinguished (d = diameter, ϕ = volume fraction):

> Ice crystals: $d = 7 - 170$ μm, on average about 50 μm, $\phi \approx 0.3$
> Lactose crystals: length ≈ 20 μm, $\phi \approx 0.005$; not always present
> Air cells: $d = 60 - 150$ μm, $\phi \approx 0.5$
> Thickness of foam lamellae: 10–20 μm
> Fat globules: $d < 2$ μm, $\phi \approx 0.06$ (including globules in clumps)
> Fat globule clumps: up to 10 μm, in size

The size of the ice crystals depends on the stirring intensity and on the cooling rate during freezing; the quicker the freezing, the smaller the crystals. Immediately after freezing, no lactose crystals are present. To be sure, the temperature is below that for saturation of lactose, as is seen in Figure 15.9, but it is still

casein micelle
fat globule
lactose crystal
ice crystal
plasma
air cell

200 μm
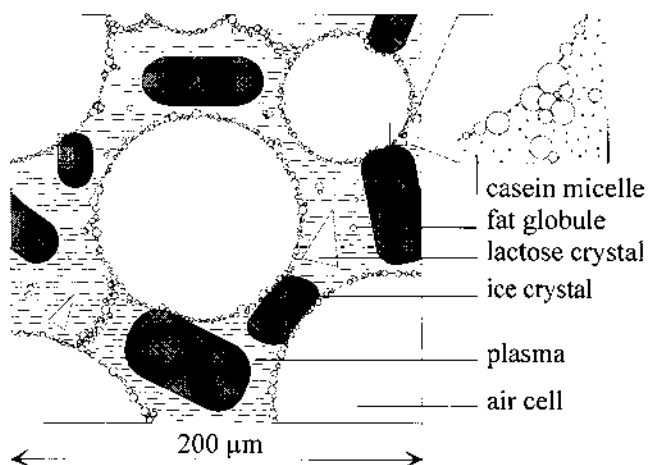
FIGURE 15.10 Schematic picture of the structure of ice cream at about $-5°C$.

above that for its homogeneous nucleation. Only after deep cooling can lactose crystals form.

The above summing up with respect to the structure does not yet complete the picture. Microscopically, many air cells can be observed to be somewhat deformed by the ice crystals, which is not surprising considering the system to be more or less completely ''filled,'' i.e., the combined volume fraction of the structural elements is about 0.8. Furthermore, the air cells are almost entirely covered with fat globules and their clumps. The clumped fat globules, together with the air cells to which they are attached, form a continuous network throughout the liquid (see Fig. 15.10). This has important effects:

a. The air cells become stabilized by the fat globules (see below).
b. After the ice crystals have melted (e.g., in the mouth) the mass retains some firmness (''stand-up''); this is illustrated in Figure 15.11, in which the extent of fat clumping is expressed as a ''churned fat index'' (which may be determined by examining which proportion of the fat creams rapidly after complete melting of the ice crystals). The stand-up is a valued organoleptic property.
c. The clumping (partial coalescence) of the fat globules changes the texture, i.e., the ice cream looks less glossy and thereby appears more
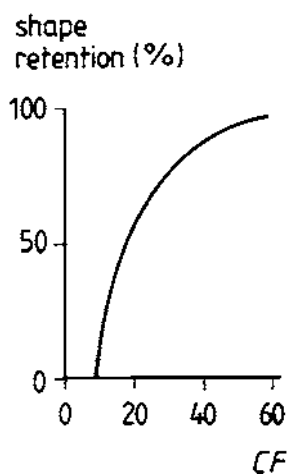
FIGURE 15.11   The influence of the extent of clumping of the fat globules (expressed as churned fat index, *CF*) on the shape retention of ice cream. The retention is the height of a cube of ice cream after keeping it for some time at room temperature, expressed as a percentage of its initial height. (From H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

attractive for most people. This property is called ''dryness,'' and it correlates very well with the experimentally obtained churned fat index.

d.   Ice cream having insufficient dryness sticks to the processing equipment, which may interfere with the packing operation, etc.

The above network of clumped fat globules is formed during freezing (see Fig. 15.12). Although the air bubbles become almost completely covered with fat globules, flotation churning presumably does not occur because too little liquid fat is available to spread over the air bubbles. In all likelihood, clumping is predominantly caused by mechanical forces, i.e., the fat globules are pushed together during beating due to the presence of ice crystals, and are damaged by them. The lower the temperature (more ice), the faster the clumping.

If unhomogenized cream would be taken, all of the fat globules together would just suffice to cover air cells of 100 μm diameter ($\phi = 0.5$) throughout. But natural milk fat globules clump rapidly, and the aggregates formed are nothing like sufficient to fully ''encapsulate'' such air cells. This implies that the cells would be unstable during and after freezing (coalescence and Ostwald ripening may occur) and large air bubbles develop, causing a coarse texture. The fat globules become much smaller in size by homogenization of the cream and then they can cover a much larger air cell surface, even after clumping (unless extensive homogenization clusters have been formed). The homogenized fat globules
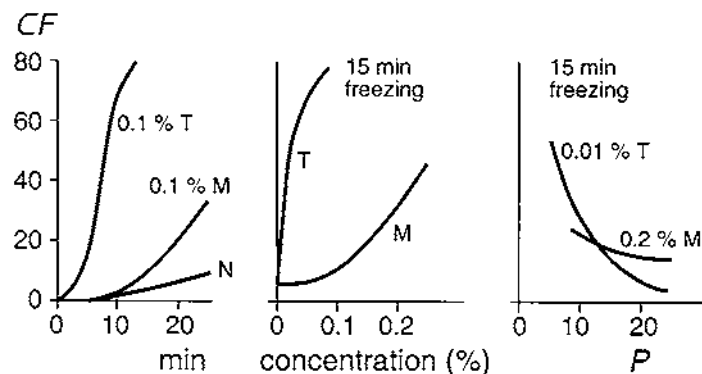
FIGURE 15.12   Influence of freezing time (min), concentration, and nature of the emulsifier and homogenizing pressure (*P*, in MPa) on the clumping of fat globules, expressed as churned fat index (*CF*). M, glycerol monostearate; T, Tween 80; N, no emulsifier. Approximate examples. (From H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.)

will, however, hardly clump, so that the desired network of clumped fat globules does not form. But these globules increasingly tend to clump if a suitable, small-molecule surfactant (usually called emulsifier) is added (Fig. 15.12). This may be explained by the emulsifier displacing part of the protein from the surface layers, which causes the fat globules to become less stable toward partial coalescence.

Obviously, the structure of the ice cream should remain intact, even on prolonged storage. The air bubbles cannot coalesce, as they have been largely immobilized. But Ostwald ripening of air bubbles, ice crystals, and lactose crystals is possible. Ostwald ripening of air bubbles can best be prevented by means of their encapsulation by fat globules. An alternative is the presence of a polysaccharide that forms a sufficiently firm gel at the low temperature and high sugar concentration applied. Ostwald ripening of ice crystals cannot be prevented unless the temperature is quite low. Concentration and viscosity of the remaining liquid are higher if the temperature is lower, and hence, the diffusion coefficient of water and of dissolved substances is smaller, causing all changes to be slow. At still lower temperature ($-30°C$?) a glassy state is attained, at which the diffusion rate of most components becomes infinitesimally slow and hence no perceptible changes occur. That is why ice cream can be kept so well at very low temperature. However, any temperature fluctuations cause a coarsening of the structural elements, mainly due to Ostwald ripening.

15.3.3 Role of the Various Components

Fat is of special importance for the flavor and for a solid structure to be formed during freezing, and hence for consistency, appearance, and melting resistance. A high fat content leads to a dry, almost grainy texture, a low fat content to a smooth, homogeneous, somewhat slimy texture.

Milk solids-not-fat contribute to the flavor. They are also responsible for part of the freezing point depression and for an increase of the viscosity. The protein partly serves to stabilize the foam lamellae during air incorporation; it is essential for the formation of fat globule membranes during homogenization. Lactose can crystallize at low temperature. The crystals formed should be small in order to prevent sandiness. To that end, cooling should be quick during freezing, and afterward temperature fluctuations should be avoided.

Sugar, often sucrose, is essential for the taste and for the freezing point depression. Too little sugar may cause too much ice to be formed; too much sugar often makes the ice cream overly sweet. To overcome this, part of the sucrose may be displaced, e.g., by glucose syrup (less sweet, greater freezing point depression per kg sugar). The sugar also causes a higher viscosity, especially when most of the water has been frozen. However, the most important role

of the sugar is that it causes far less water to freeze than otherwise would be the case. As a result, the consistency of the ice cream is softer and its mouthfeel less cold.

The role of the *''stabilizer''* or, more properly speaking, of the thickening agent is not quite clear. Among those used are gelatin, alginate, carrageenan, pectin, locust bean gum, guar gum, xanthane, carboxymethylcellulose, and mixtures. Of course, these substances affect the consistency and hence, for instance, the heat transfer during the freezing. If little clumping of fat globules occurs as, for instance, in low-fat ices, the desired firmness and prevention of excessive Ostwald ripening of air bubbles must be achieved by means of thickening agents; but these agents may cause the consistency of the product to become somewhat slimy in the mouth. Furthermore, the thickening agents are often assumed to counteract the Ostwald ripening of ice and lactose crystals, and even to prevent crystallization of lactose. Many thickening agents at high concentrations (as is the case in ice cream at low temperature) do indeed lower the crystallization rate and thereby slow down Ostwald ripening; but it is very unlikely that they can inhibit crystallization.

Emulsifier is not needed in the proper sense of the word (more than sufficient protein is present during homogenization) and it does not play a significant role in foam formation. It serves to stimulate the fat globules to clump. The emulsifiers used include egg yolk, monoglycerides, poly(oxyethylene) sorbitan esters (Tweens), and esters from citric acid and monoglycerides.

Flavoring agents are self-evident. Sometimes an antioxidant is added.

Naturally, *ice crystals* are essential for the consistency and for the coolness in the mouth. Moreover, the low temperature causes the sweetness to be less intense. The crystals should be not too large; hence, freezing should be fast and the storage temperature should not fluctuate.

Air cells play a threefold part. They make the ice cream light; otherwise it would be too rich. They soften its consistency and thereby make it deformable in the mouth. They moderate the coldness by lowering the rate of heat transfer; otherwise the ice cream would be far too cold in the mouth. The amount of air may be bound to a maximum since, according to statutory requirements, the density of the ready-made ice cream may not be below a given value, say, 0.5.

SUGGESTED LITERATURE

- Aspects of coffee cream, whipping cream, and ice cream are discussed by:
   H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.
- A practical book about ice cream is:
   R. T. Marshall and W. S. Arbuckle, *Ice Cream*, 5th ed., Chapman and Hall, New York, 1996.

16

Concentrated Milks

16.1 EVAPORATED MILK

16.1.1 Description

Evaporated milk is sterilized, concentrated, homogenized milk. The product has a long shelf-life (it can be kept for several months, even at tropical temperatures), is completely safe for the user, and can be kept without refrigeration. After dilution, flavor and nutritive value of the product are not greatly different from that of fresh milk. Traditionally, sterilization occurs in cans or bottles. Currently, ultra-high temperature short-time heated (UHT) heating is also applied, followed by aseptic packing in cardboard containers. A major problem with sterilization is the heat stability; the higher the concentration of the milk, the lower its stability. That is why concentrating cannot be by more than about 2.6 times, which implies about 22% solids-not-fat in the evaporated milk.

The traditional product shows browning because of Maillard reactions, and it also has a ''sterilized'' flavor. The sterilization can destroy up to 10% of the available lysine, about half of vitamins B_1, B_{12}, and C, and smaller proportions of vitamin B_6 and folic acid. The product is quite viscous; its viscosity amounts to 40 mPa · s or about 20 times that of fresh milk. These disadvantages do not apply to UHT evaporated milk, wherein the loss of nutrients is far smaller and there is a whiter color, a lower viscosity, and a better flavor.

Evaporated milk was mainly used in countries with little or no milk production, especially in the tropics; it is generally diluted with water before use. An alternative is to make recombined milk from skim milk powder, anhydrous milk fat, and water. Currently, evaporated milk is used in coffee in certain countries. It can be added while cold because a fairly small amount is involved, as compared

to nonevaporated milk. After the bottle has been opened the milk can be kept in the refrigerator for up to 10 days because it initially contains no bacteria at all and because contaminating bacteria grow somewhat more slowly due to the reduced water activity, which is about 0.98.

Table 16.1 gives the composition of some kinds of evaporated milk and skim milk. In order to control the heat stability of the milk, $CaCl_2$ or sodium carbonates, phosphates, or citrates may be added. Sometimes, a thickening agent (e.g., 0.015% carrageenan) is added to slow down creaming.

16.1.2 Manufacture

Figure 16.1 outlines manufacturing processes of in-bottle and UHT sterilized whole evaporated milk. Several variations are possible. Some process steps are discussed in more detail.

Preheating serves to enhance the heat stability of the evaporated milk (see Section 16.1.4), to inactivate enzymes, and to kill microorganisms as well as many bacterial spores. The heating temperature-time relationship is usually selected on the basis of heat stability. Formerly, a long heat treatment (e.g., 20 min) at a temperature below 100°C was often applied. Currently, UHT treatment is generally preferred. It reduces the number of spores in the milk considerably and therefore a less intensive sterilization suffices.

Concentrating. The milk is usually concentrated by evaporation (see Section 9.2). Standardization to a desired dry matter content is of much concern. A higher concentration causes a lower yield and a poorer heat stability. Continuous standardization is usually applied by determining the mass density. Based on that parameter either the raw milk supply or the steam supply is adjusted; it is obvious that density and dry matter content of the raw milk must be determined. Alternatively, standardization can be based on refractive index determination. The milk can also be concentrated by reverse osmosis (see Section 9.4).

After concentration, the manufacturing processes for in-bottle sterilized and UHT sterilized evaporated milk differ. The former process is discussed first.

TABLE 16.1 Approximate Composition of Some Kinds of Evaporated Milk

Type	Fat (%)	Solids-not-fat (%)	Concentration factor
Evaporated milk, American standard	7.8	18.1	2.1
Evaporated milk, British standard	9	22	2.6
Low-fat evaporated milk	4	20	2.25
Evaporated skim milk	0.1	22	2.35
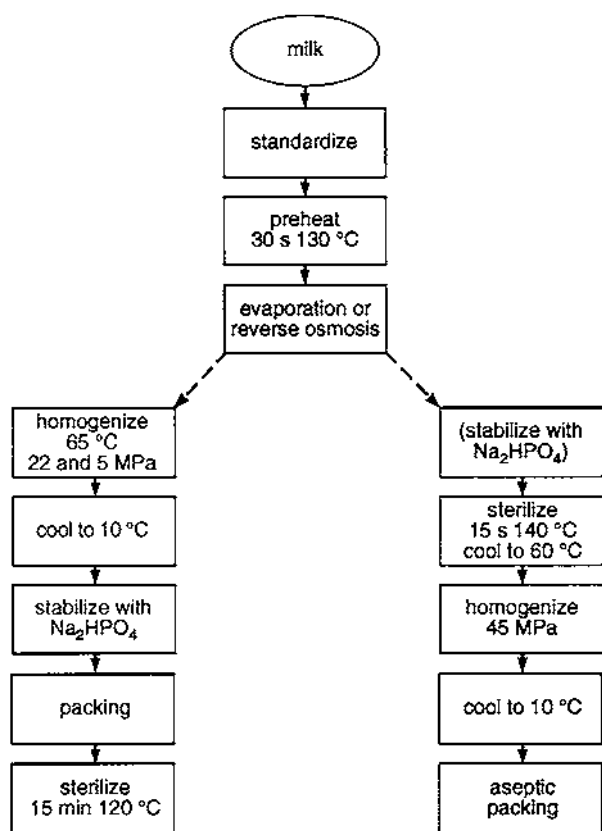
FIGURE 16.1 Examples of the manufacture of in-bottle (left) and UHT (right) sterilized, evaporated whole milk.

Homogenization serves to prevent creaming and coalescence. It should not be too intensive because the heat stability becomes too low.

Stabilization. To ensure that the evaporated, homogenized milk does not coagulate during sterilization and at the same time does acquire a desirable viscosity, a series of sterilization tests is often done on small quantities of the evaporated milk to which varying amounts of a stabilizing salt (for the most part Na_2HPO_4) are added. The tests are needed because variation occurs among batches of milk. Essentially, the addition of the salt means adjusting the pH (see Section 16.1.4). Because further processing must be postponed until the test results are available, this necessitates cooling the evaporated milk after its homogenization and keeping it for a while. Long-term storage should be avoided to pre-

vent bacterial growth; moreover, cold storage of the milk increases the tendency of age thickening (see Section 16.1.6). The stabilizing salt is added as an aqueous solution, which dilutes the evaporated milk slightly. Therefore, the milk is often concentrated somewhat too far and restandardized to the correct dry matter content during ''stabilization.''

Packing in cans is common. The tin plate of the cans is coated (provided with a protective layer of a suitable polymer) to prevent iron and tin from dissolving in the product. After filling, the cans may be soldered up, but mechanical sealing is currently preferred. Evaporated milk intended for use in coffee is usually packed in bottles that are closed with a crown cork.

Sterilization. In-bottle or in-can sterilization can be applied batchwise (in an autoclave) or continuously. Machines that have rotary air locks (to maintain the pressure) may be applied for cans and hydrostatic sterilizers for bottles (see Fig. 6.20).

The sterilization is primarily aimed at killing all bacterial spores—reduction to, say, 10^{-8} spores per ml—and inactivating plasmin, i.e., milk proteinase. Lipases and proteinases from psychrotrophs should be absent from the raw milk because these enzymes would be insufficiently inactivated. The most heat-resistant spores are those from *Bacillus stearothermophilus*. This bacterium does not grow at moderate temperatures but may do so in the tropics. D_{121} of the spores is some 4–7 min. The preheating as given in Figure 16.1 thus suffices for a sterilizing effect $S \approx 1$, whereas the sterilization gives $S \approx 3$ at most and, hence, added together $S \leq 4$. Contamination by these spores should therefore be slight and growth of the organism occurring in the evaporator, possibly followed by sporulation (e.g., during intermediate cold storage), should rigorously be avoided; see also Section 17.3. If the sterilizing effect is adequate for *B. stearothermophilus*, then *B. subtilis, Clostridium botulinum*, and *C. perfringens* are also absent (see Table 6.4).

UHT sterilization kills bacterial spores more effectively than in-bottle sterilization. The combination of preheating and UHT treatment of the concentrate as shown in Figure 16.1 suffices to inactivate plasmin. The preheating is also required to prevent excessive heat coagulation in and fouling of the UHT sterilizer. Some heat coagulation nearly always occurs, and the subsequent homogenization is also meant to reduce the size of the protein aggregates formed. Aseptic homogenization must be applied. Indirect UHT sterilization in a tubular heat exchanger allows the pump of the homogenizer to be fitted before the heater and the homogenizing valve behind it. Thereby the risk of recontamination is diminished. The addition of stabilizing salt can often be omitted if UHT sterilization is applied or if the amount to be added is not so critical that sterilization tests must be carried out. It implies that the whole process from preheating up to and including aseptic packing can proceed without interruption. Aseptic packing and suitable packing materials are discussed in Chapter 13.

Recombination. Manufacture of evaporated milk by means of recombination is briefly outlined in Figure 16.2. The skim milk powder used has to comply with strict requirements. The powder must have been made from skim milk that is heated so intensely (e.g., 1 min at 130°C) that the recombined concentrated milk after its homogenization is sufficiently heat-stable. Spores of *B. stearothermophilus* should largely be absent, so that a somewhat more moderate sterilization of the evaporated milk suffices. Sometimes, up to 10% of the skim milk powder is displaced by sweet cream buttermilk powder to improve the flavor of the product. The copper and peroxide contents of the anhydrous milk fat should
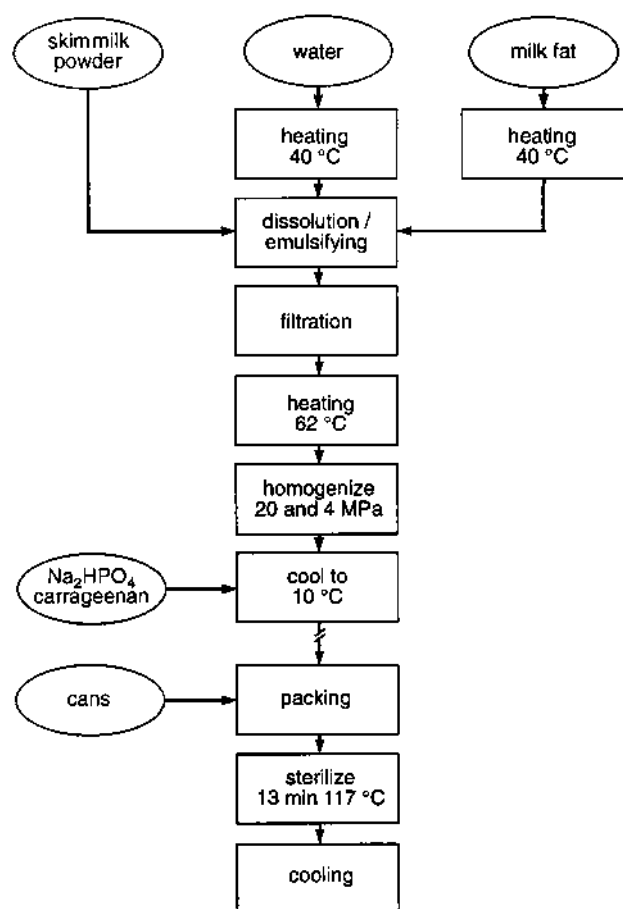
FIGURE 16.2 Example of the manufacture of recombined evaporated milk.

be low to avoid flavor deterioration. A high level of calcium in the water used can cause problems with heat stability.

''Filled evaporated milk'' is also made. A fat different from milk fat is used.

16.1.3 Organoleptic Properties

Maillard reactions are paramount for flavor and color of evaporated milk. Obviously, temperature and duration of the heat treatment during manufacture determine the initial concentration of the reaction products, but ongoing Maillard reactions occur during storage, especially at a high temperature (see Fig. 16.3). The milk eventually develops a stale flavor, also due to Maillard reactions. The flavor after a long storage time differs considerably from that directly after intense heating. This is because the complicated set of reactions involved leads to different reaction products at different temperatures. A ''sterilized flavor'' may be appreciated when the milk is used in coffee. Off-flavors due to autoxidation need not occur.

When the milk is used in coffee the brown colour is often desirable, to prevent the coffee's acquiring a grayish hue. The brown color depends greatly on the Maillard reactions, though the color of the fat is also involved.

The viscosity of evaporated milk is often considered an important quality mark. Many consumers prefer the milk to be viscous. This can be achieved by sterilization in such a way that visible heat coagulation is barely prevented. UHT evaporated milk is always less viscous and, therefore, κ-carrageenan is sometimes added (see Section 16.1.6).
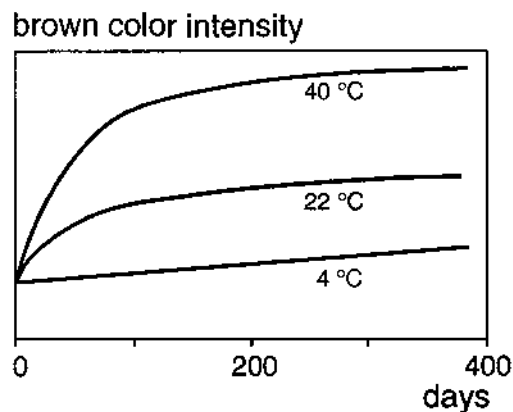
FIGURE 16.3 Browning (arbitrary units) during storage of evaporated milk at various temperatures. After S. Patton, *J. Dairy Sci.* **35** (1952) 1053.

If the original milk contains detectable bacterial lipases and proteinases, these enzymes may remain active in the evaporated milk and lead to strong deterioration, i.e., soapy-rancid and bitter flavors, and to age thinning. Evaporated skim milk may even become more or less transparent due to proteinase activity.

16.1.4 Heat Stability

The mechanisms of heat coagulation of milk and the factors affecting the heat coagulation time (HCT) are discussed in Section 6.2. As mentioned, concentrated milk is far less stable during sterilization than nonevaporated milk and the fairly intensive homogenization applied decreases the heat stability further. Moreover, evaporated milk should increase in viscosity during sterilization. Essentially, the viscosity increases by incipient coagulation. A subtle process optimization is needed to meet these requirements.

In any case, the milk must be preheated before evaporation in such a way that most serum proteins are denatured (see Fig. 6.9E). Otherwise the evaporated milk forms a gel during sterilization due to its high concentration of serum proteins. Preheating is, for example, 3 min at 120°C. Figure 16.4 shows the significant effect of preheating.

The pH should always be adjusted. Preheating and evaporation have low-
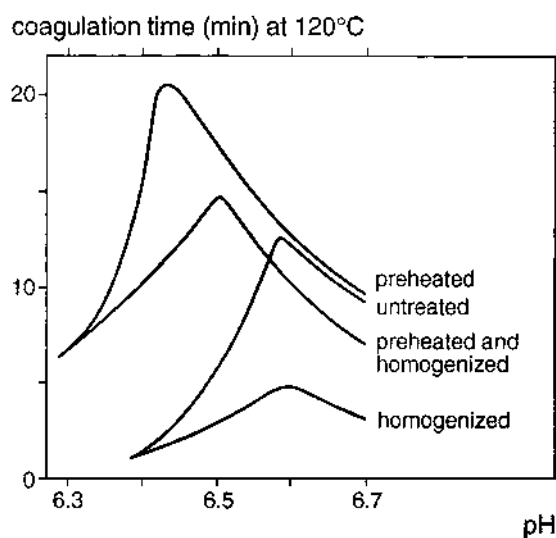
FIGURE 16.4 Influence of preheating and homogenization on the heat stability of evaporated milk (British standard) as a function of its pH (measured at room temperature before sterilization). Approximate examples.

ered the pH to about 6.2 (American standard) or 6.1 (British standard), and that is clearly below the optimum pH. In practice, $Na_2HPO_4 \cdot 12H_2O$ is usually added, but NaOH can also be used.

The effect of homogenization is also illustrated in Figure 16.4 (see also Section 8.6). The influence of some variables like the homogenization temperature differs from that in sterilized cream (Section 15.1). Homogenization of evaporated milk does not lead to formation of homogenization clusters. It is often observed that a slight homogenization increases HCT (Fig. 16.5), which cannot be easily explained.

Figure 16.5 summarizes the approximate effect of several factors on the heat stability. Clearly, UHT heating of evaporated milk after homogenization is not possible. Even traditional sterilization is difficult if the milk is highly concentrated or if the evaporated milk is intensely homogenized. There are some other variables. The heat stability can be improved by lowering the calcium content of the milk before evaporation by means of ion exchange. Addition of 15 mmol H_2O_2 (0.05%) or of about 15 μmol Cu^{2+} (0.5–1 mg · kg^{-1}) after preheating but before evaporation increases the heat stability.

16.1.5   Creaming

Creaming of evaporated milk eventually leads to formation of a solid cream plug that cannot be redispersed. Partial coalescence or bridging of adjacent fat globules due to ''fusion'' of the fragments of casein micelles in their surface layers may be responsible. Accordingly, intensive homogenization is necessary (see Chapter 8).

The newly formed surface layers during homogenization can be fairly thick. The preheating has left hardly any dissolved serum proteins and the evaporation
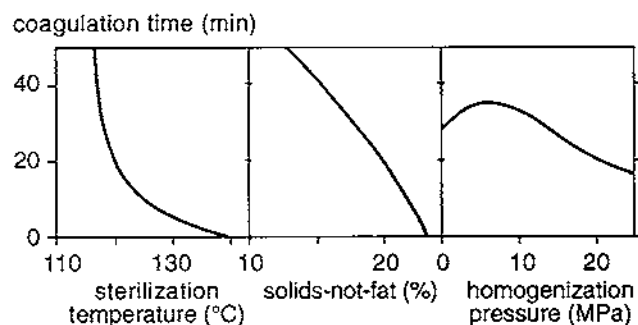
FIGURE 16.5   Heat stability, measured at 120°C (unless otherwise stated) and at the optimum pH of preheated homogenized evaporated milk. Approximate examples of the influence of some variables.

and sterilization steps have increased the average diameter of the casein micelles. Especially after homogenization at high pressure and low temperature, the layers may be thick enough for the globules to have a higher density than the plasma; consequently, they sediment rather than cream. As a result, the fat content of evaporated milk in both the top and bottom layers of a can that has been stored undisturbed for several months is often found to be higher than that in the middle.

A higher viscosity of the evaporated milk often involves a slower creaming, but the relations are not straightforward. To begin with, it is the viscosity of the plasma phase, not that of the product, that determines creaming rate. Generally, a high viscosity is due to an approaching heat coagulation. The homogenized fat globules tend to participate in this coagulation, hence to form clusters that would cream rapidly. In fact, measures that counteract the heat coagulation, such as the addition of traces of copper (Section 16.1.4), usually lead to a decreased creaming, despite the lower viscosity resulting from such additions. In any case, the creaming in evaporated milk has been insufficiently investigated. The viscosity of the plasma at the very low shear rates relevant for creaming is not known and it has not yet been established under what conditions evaporated milk can attain a yield stress. The fat reaching the cream layer in 1 month, as calculated from Table 8.3, amounts to some percentage of the total fat involved and that figure agrees roughly with experimental data, but the creaming can be faster at a higher temperature.

As already stated, homogenization has an adverse effect on the heat stability and, consequently, the homogenization pressure cannot be high. It is especially the largest fat globules that exhibit creaming, and it is therefore advisable to aim at having the relative width (c_s) of the globule size distribution as small as possible. The width is greatly affected by the type of homogenizer applied. Two-stage homogenization is often used, but its effect on c_s is negligible (neither is this type of homogenization required to break up homogenization clusters because these are not formed). Homogenizing twice does lead to a lower c_s. Alternatively, if need be, the lightly homogenized evaporated milk may be separated by centrifugation and depleted of the largest fat globules. The cream obtained can be added to the unhomogenized concentrate.

UHT evaporated milk can be homogenized far more intensely because the sterilization precedes the homogenization. Intense homogenization is also required to prevent excessive creaming because the viscosity of the plasma phase is much lower than in conventional evaporated milk.

16.1.6 Age Thickening and Gelation

When evaporated milk is kept, its viscosity may initially decrease slightly (Fig. 16.6). This may be explained in terms of casein micelle aggregates changing from an irregular to a spherical shape, as a result of which the effective volume

apparent viscosity (mPa·s)
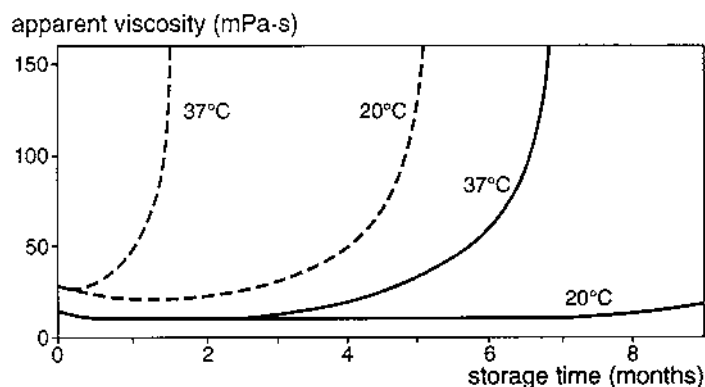
FIGURE 16.6 Age thickening of evaporated milk at two temperatures. Approximate examples for UHT evaporated milk. Polyphosphate added (——) or not (---). After P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

fraction decreases (see Fig. 16.7, i.e., the change from A to B). Subsequently, the viscosity tends to increase, and it becomes dependent on the shear rate (hence, it is an apparent viscosity). Soon the milk displays a yield stress and a gel is formed that firms rapidly. (The subject is briefly mentioned in Section 3.2.) The mechanism involved is not quite clear. In most cases it is not caused by proteolytic enzymes; neither are Maillard reactions responsible, though the latter parallel gelation. Moreover, gelation is not related to heat coagulation. For instance, it does not depend significantly on the pH and its rate increases rather than decreases after lowering of the calcium content. Electron microscopy reveals that thread-like protrusions appear on the casein micelles, which eventually form a
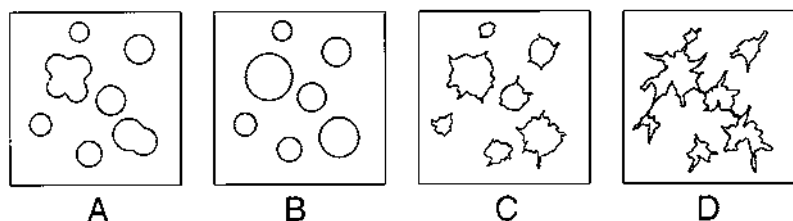
FIGURE 16.7 Schematic picture of the change observed in the casein micelles of evaporated milk during storage. The apparent viscosity is at a minimum in stage B.

network. This is illustrated in Figure 16.7, frames C and D. It is likely that a slow change in the micellar calcium phosphate is at least partly responsible for the changes observed, but a definitive explanation is still lacking.

Age thickening and gelation tend to occur fast in UHT evaporated milk. It may then be due to proteolysis caused by enzymes released by psychrotrophs, but also if such enzymes are absent, fast age gelation occurs. A more intense sterilization after evaporation delays gelation. Gelation is faster in a more concentrated milk (cf. Fig. 9.9C) and at a higher storage temperature (Fig. 16.6). Addition of sodium polyphosphate (about 0.4% in the dry matter) delays gelation considerably; the higher the molar mass of the phosphate, the more effective it is. Addition of citrate or orthophosphate often accelerates gelation, presumably because of binding of calcium. Polyphosphates may be hydrolyzed to yield orthophosphate, especially during heating. Consequently, addition of polyphosphate does not counteract gelation of in-bottle sterilized evaporated milk, to the contrary.

Conventional evaporated milk only gels if kept for a long time at a high temperature (as in the tropics). Extensive Maillard reactions then occur also. Rapid gelation can occur, however, if the evaporated milk before its sterilization is kept refrigerated at, say, 4°C for a few days.

All in all, adequate measures can be taken to delay gelation of the evaporated milk for a considerable time. Gelation can be examined by suspending a can of evaporated milk on a torsion wire and checking whether the milk has elastic properties. If so, the can will keep oscillating for a while when it is given a turn and then released.

16.2 SWEETENED CONDENSED MILK

16.2.1 Description

Sweetened condensed milk is milk that is concentrated by evaporation, to which sucrose is added to form an almost saturated sugar solution, after which it is canned. The product was already known before milk could be sterilized or dried at moderate temperature. It thus constitutes one of the oldest milk preserves, dating from 1856. The high sugar concentration is primarily responsible for the keeping quality of the product and for its fairly long shelf life, even after the can has been opened, though it then will eventually become moldy.

Table 16.2 gives compositions; sweetened condensed skim milk is also made. The milk is remarkably concentrated: the mass concentration ratio, Q, equals 4.6–5, and the increase in concentration relative to water, Q^*, is 7.3–8.5. Because of this and the high sugar content, the product is highly viscous: $\eta' \approx$ 2 Pa $\cdot$ s, i.e., about 1000 times the viscosity of milk. The product is somewhat glassy in appearance because the fat globules show little light scattering. The

TABLE 16.2   Approximate Composition of Two Kinds of Sweetened
Condensed Milk

	American standard	British standard
Fat content (%)	8	9
Milk solids-not-fat (%)	20	22
Lactose (%)	10.3	11.4
Sucrose (%)	45	43.5
Water (%)	27	25.5
g lactose/100 g water	38.3	44.6
g sucrose/100 g water	167	171
Concentration factor Q	4.60	5.00

high concentration of dissolved substances causes the refractive index of the continuous phase to almost equal that of fat. For the same reason, light scattering due to the casein micelles is also low. The turbidity of the product is largely due to lactose crystals. A large part of the lactose crystallizes because of its supersaturation.

Sweetened condensed milk is used in coffee, tea, and cooking, especially in the tropics. The product is sometimes used (after dilution) as baby food, but that must be strongly discouraged for nutritional reasons. Although alternative products like recombined beverage milk are available in many tropical countries, sweetened condensed milk is still used. It can also be made locally by recombination; sometimes, ''filled'' sweetened condensed milk is made.

In some countries, a product containing less sugar (more than 70 g sucrose per 100 g water) is made for use in chocolate and candy manufacturing. The product may be kept refrigerated for a limited time.

16.2.2   Manufacture

Figure 16.8 is a flow diagram of a typical manufacturing process for sweetened condensed milk. Several variants of the process are possible. Most of the process steps will be discussed briefly.

Heating. Pathogens and potential spoilage organisms must be killed. Among the enzymes, milk lipase should primarily be inactivated; bacterial lipases are not inactivated and, if present, can cause severe deterioration. Deterioration caused by proteinases has not been reported. The heating intensity considerably affects viscosity, age thickening, and gelation of the product (Section 16.2.4), so the actual heat treatment must be adjusted to these properties. UHT treatment at about 130–140°C is commonly applied.
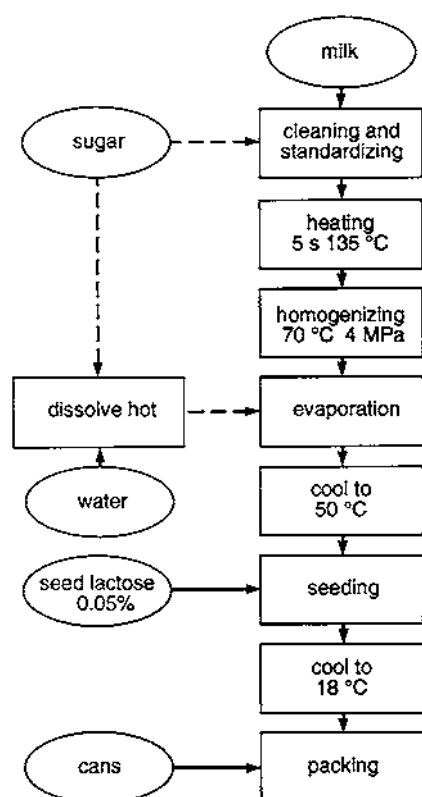
FIGURE 16.8 Example of the manufacture of sweetened condensed milk.

Homogenization. Creaming is often not a big problem and therefore homogenization is not always applied. Currently, however, sweetened condensed milk is made less viscous (and exhibits less thickening) than previously. The mass density difference between fat globules and continuous phase is large, over $400 \text{ kg} \cdot \text{m}^{-3}$; if the effective viscosity of the continuous phase is taken to be 1 $Pa \cdot s$ and if homogenization is omitted, the creaming rate would be about 1% of the fat per day. This is too high, so that homogenization is often applied, though at low pressure, i.e., 2–6 MPa. Homogenization also increases the viscosity of the product slightly.

Sugar can simply be added to the original milk. The amount added can be adjusted readily and accurately, and the sugar is pasteurized along with the milk. However, this procedure causes fairly extensive Maillard reactions during heating

and evaporation, and above all a faster age thickening. Apart from that, evaporation is more difficult (especially when a multiple-effect evaporator is used) because of a further increase in boiling point and a decreased coefficient of heat transmission (due to the higher viscosity). Alternatively, a concentrated sugar solution, which should be sufficiently heat-treated to kill any osmophilic yeasts, is added at the end of the evaporation step. The sugar involved should be refined and should be devoid of invert sugar to prevent excessive Maillard reactions.

Concentration is usually done by evaporation, but reverse osmosis can also be used. A falling film evaporator is usually used to remove the bulk of the water and another evaporator to remove the remainder. The latter is usually a conventional rising film evaporator with separate steam supply, in which part of the product recirculates to ensure that the highly viscous sweetened condensed milk keeps the heating surface covered. To achieve that, somewhat higher temperatures (up to 80°C) are often applied, which implies a lower viscosity in the evaporator but a higher initial viscosity of the final cooled product.

The low water content of the sweetened condensed milk implies high viscosity and boiling point. Evaporation in continuously operating equipment with many effects is therefore not easy (the steam management is thus less economical). Fouling thus readily occurs. It is hard to accurately adjust the desired water content, which is mostly monitored by means of refractive index. This method can be effectively used because sweetened condensed milk is hardly turbid.

Cooling and seeding. In these steps, formation of large lactose crystals must be avoided (Section 16.2.5). Consequently, seed lactose is added. Before that, the condensed milk must be cooled to a temperature at which lactose is supersaturated so that the seed lactose does not dissolve. However, the temperature must not be so low that spontaneous nucleation can occur before the seed crystals are mixed in. After seeding, cooling should be continued to crystallize the lactose. In a continuous process, the cooling should be fast, but that is by no means easy for such a highly viscous product. A vacuum cooler is often used in which a thin layer of the milk passes the wall of a vat that is under vacuum. Cooling from 50°C to 18°C causes some 3 kg of water to evaporate from 100 kg of sweetened condensed milk, which needs to be taken account of during evaporation. Alternatively, the sweetened condensed milk can be cooled in a scraped-surface heat exchanger.

Packing in cans is common. The cans are then covered with a lid and the seams sealed. Cans and lids are first sterilized, e.g., by flaming. The packing section is supplied with air purified through bacterial filters. In this section, rigorous hygienic standards are paramount.

The manufacture of sweetened condensed milk via recombination has no specific details. Medium-heat skim milk powder is usually used to achieve the desired viscosity.

16.2.3 Microbial Spoilage

Sweetened condensed milk is not sterile. It contains living bacteria and spores. The low water activity (about 0.83) or, rather, the high sugar content prohibits growth of most but not all microorganisms.

a. Deterioration usually occurs by osmophilic yeasts, most of which belong to the genus *Torulopsis*. The yeasts often cause gas formation (bulging cans), a fruity flavor, and coagulation of protein. Coagulation may result from ethanol production. As a result, the product becomes unacceptable. The yeasts do not start easily, especially if the sugar concentration is high. It may thus take several weeks for incipient growth to be perceptible.
b. Some micrococci may grow in sweetened condensed milk, though slowly, especially if water activity and temperature are high. Presumably, the presence of oxygen is required. It may happen that they grow to reach a colony count of, say, 10^5 ml^{-1} and then stop growing, without causing noticeable defects. If they keep growing, aggregates eventually form and several off-flavors develop.
c. Some molds, especially strains of *Aspergillus repens* and *A. glaucus*, can grow as long as oxygen is present. If so, fairly firm colored lumps are formed and an off-flavor develops. One spore in one air bubble can cause such a lump.

Obvious remedies for microbial spoilage include the killing of all saprophytes and mold spores in the milk and in the sugar. No single bacterial spore can germinate in sweetened condensed milk. Growth of harmful microorganisms in the dairy plant should be rigorously avoided. No sugar and residues of the milk should be left around. Satisfactory hygienic standards must therefore be maintained, especially in the packing section. No microorganisms harmful for the product can grow during concentration in the evaporator, but immediately after evaporation the machinery must be thoroughly cleaned. Any mold spores can be removed by air filtration.

The packing machine has to comply with special requirements. It should fill the cans very accurately with a safety margin of 1 g. Too little condensed milk in the cans means that more air is left, which increases the chance of growth of molds and micrococci. If the cans are overfilled, the milk may spill over the side and encourage growth of osmophilic yeasts.

The microbial composition and the tendency to thicken with age of the bulk of the sweetened condensed milk can be checked by means of shelf life tests. Several cans are kept for a few weeks at high temperature, say 35°C, and subsequently examined for growth and other parameters. However, complete certainty is not obtained.

TABLE 16.3 Apparent Viscosity (Pa · s) of Sweetened Condensed Skim Milk at 16°C as a Function of Preheating and Storage Time

Preheating		Viscosity after		
Temp. (°C)	Time (min)	1 day	24 days	58 days
71	10	2.5	4	10
82	10	36	53	85
95	10	57	85	(gel)
115.5	0.5	2	3	7

After B. H. Webb and C. F. Hufnagel, U.S. Dept. Agri. Bur. Dairy Ind., Inf. 47(1947).

16.2.4 Chemical Deterioration

The main change in sweetened condensed milk during storage is presumably *age thickening* and, finally, *gelation* (see also Section 16.1.6). Sweetened condensed milk is far more concentrated than evaporated milk. Nevertheless, it does not thicken markedly faster with age. It is usually assumed that added sucrose inhibits age thickening; other sugars or hexitols have a similar effect. An important difference from evaporated milk is the far higher viscosity of the continuous phase of sweetened condensed milk. It means that the diffusion coefficients are smaller and that all diffusion-limited reactions are slower. Sucrose increases the Ca^{2+} activity. Another difference with evaporated milk is that an initial decrease in viscosity before age thickening is not observed, but that need not be surprising because sweetened condensed milk would contain no flocculated casein micelles. The viscosity η' increases almost linearly with time. Various research results on age gelation tend to agree poorly with one another because thickened sweetened condensed milk shows considerable shear rate thinning, and the effective shear rate during the viscosity measurement is often not reported.

The following are the main factors affecting age thickening:

a. *Kind of milk*. Variation occurs among batches of milk, often with an effect of season. Milk of cows in early lactation may be more sensitive.
b. *Preheating of milk*. Table 16.3 gives some examples. Also longer heating times at UHT temperatures lead to little age thickening. So UHT heating is now generally applied. The heating affects the initial viscosity considerably (Table 16.3) and that will be the main effect, i.e., the lower the initial viscosity, the lower the volume fraction of casein particles, and the longer the elapsed time before a gel is formed (assuming the volume fraction of the casein particles increases at the same relative rate).

c. *Stage at which sugar is added*. The later in the evaporating process, the less the age thickening.
d. The higher the *concentration factor,* the more the age thickening. That explains why sweetened condensed milk of the British standard thickens faster with age than that of the American standard.
e. *Stabilizing salts*. The influence of added salts varies widely and depends on, for example, the stage at which it is added. Salts are added up to, say, 0.2%. Adding a small amount of sodium tetrapolyphosphate (e.g., 0.03%) mostly delays age thickening considerably, whereas adding more often has the opposite effect.
f. Age thickening considerably increases with storage temperature: $Q_{10} \approx$ 3.4. At tropical temperatures, gelation inevitably occurs within about 1 year.

Ongoing *Maillard reactions* are likewise inevitable. Brown discoloration is stronger as the storage temperature is higher, as the milk is evaporated to a higher concentration, and as more intense heating is applied. Additional Maillard reactions occur if the added sucrose contains invert sugar. Adding the sugar before evaporation causes faster browning than does adding it afterward. The long-term exposure of the product to relatively high temperatures may cause hydrolysis (inverting) of sucrose and hence formation of reducing sugars. Faster browning may also be caused by the activity coefficient of lactose being increased by the high sucrose concentration.

   Autoxidation of fat can occur because the packed product contains a little oxygen and has often not been heated sufficiently for antioxidants to be formed. Obviously, any copper contamination should be rigorously avoided.

   Enzymic deterioration is discussed in Section 16.2.2.

16.2.5 Lactose Crystals

Sweetened condensed milk contains around 38–45 g lactose per 100 g water, as shown in Table 16.2 (leaving water nonsolvent for lactose aside). Figure 2.3 illustrates the solubility of lactose at room temperature to be about 20 g per 100 g water, but in sweetened condensed milk the solubility is about half as large due to the presence of sucrose (see Fig. 16.9). It implies that 75% of the lactose tends to crystallize, meaning about 7.5 g per 100 g sweetened condensed milk. Figure 2.3 shows also that homogeneous nucleation would hardly occur at room temperature and slightly over because the supersaturation is too slight. In other words, heterogeneous slow nucleation occurs and therefore only a few nuclei would be formed per unit volume of milk, leading to large crystals.

   Without special measures, sweetened condensed milk would obtain a relatively large quantity of lactose in the shape of large crystals. These crystals sedi-

relative solubility
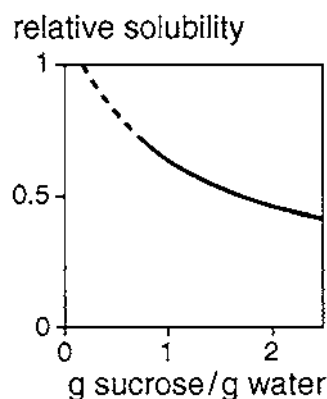
FIGURE 16.9   Influence of sucrose on the relative solubility of lactose (per unit mass of water). Approximate results at about 40°C.

ment (after sedimentation an even distribution throughout the viscous liquid is hard to achieve) and are responsible for a sandy mouth-feel. Although the crystals are not so large as to be felt singly in the mouth, they can be large enough to cause a nonsmooth impression. To avoid this, they should be smaller than about 8 μm in length.

Preventing crystallization is not possible and, accordingly, a large number of crystals should be the goal. This may be achieved by using an ingenious cooling schedule, but fully satisfactory results can only be obtained by using seed lactose. Adding 0.03% seed lactose represents 0.004 time the amount of lactose to be crystallized. The final size of the crystals in the product should not exceed 8 μm. Consequently, the seed lactose would contain enough seed crystals (one per crystal to be formed) if its crystal size does not exceed about $(0.004 \times 8^3)^{1/3} = 1.25$ μm. Such tiny crystals can be made by intensive grinding of lactose, which is crystallized as usual. Another method is as follows: A lactose solution is spray-dried. Then the amorphous lactose powder is left to absorb just enough water (at $a_w \approx 0.4$) to allow crystallization, which gives extremely small crystals (<1 μm length) embedded in amorphous lactose. Grinding readily pulverizes the powder into separate crystals.

In addition to lactose crystals, sucrose crystals can also be formed if too much sucrose has been added or the milk has been concentrated too far, and the sweetened condensed milk is kept at a low temperature. Since the supersaturation of sucrose would be slight, large crystals are formed that make the product definitely sandy.

SUGGESTED LITERATURE

- A general overview, on an introductory level, is given by:

    M. Carić, *Concentrated and Dried Dairy Products*, VCH, New York, 1994.
- Aspects of heat coagulation and age gelation are discussed in:

    P. F. Fox, ed., *Advanced Dairy Chemistry,* Vol. 1, *Proteins*, Elsevier, London, 1992, Chapters 15 (H. Sing and L. K. Creamer, ''Heat stability of milk'') and 17 (V. R. Harwalker, ''Age gelation of sterilized milks'').

17

Milk Powder

In this chapter attention is paid to spray-dried milk powder (i.e., powder made from milk with a normal fat content), to skim milk powder, and, to a lesser extent, to whey powder. We restrict ourselves to spray-dried products, except for a small section. Basic principles of evaporation and drying are described in Chapter 9.

17.1 OBJECTIVES

We may distinguish the following objectives:

a. The main purpose of the manufacture of milk powder is to convert the liquid perishable raw material to a product that can be stored without substantial loss of quality, preferably for some years. Decrease in quality mainly concerns formation of gluey and tallowy flavors (due to Maillard reactions and autoxidation, respectively) and decreasing nutritive value (especially decrease in available lysine). If the water content becomes very high and the storage temperature is high, caking (due to lactose crystallization) and enzymic and even microbial deterioration can occur; however, such problems need not occur.

b. The powder should be easy to handle. It should not dust too much or be overly voluminous. It should be free-flowing, i.e., flow readily from an opening, and not stick to the walls of vessels and machinery. The latter requirement is of special importance for powder used in coffee machines, etc.

c. After adding water the powder should be reconstituted completely and readily to a homogeneous mixture, similar in composition to the origi-

nal product. Complete reconstitution means that no undissolved pieces or flakes are left and that neither butter grains nor oil droplets appear on top of the solution. ''Readily reconstituted'' means that during mixing of powder and water no lumps are formed because these are hard to dissolve. In the ideal situation the powder will disperse rapidly when scattered on cold water; this is called ''instant powder.'' Special processing steps are needed to attain this property (''instantizing''). The importance of instant properties closely depends on the kind of application. They are paramount for use in the household, e.g., to reconstitute the powder to milk for liquid consumption. In a plant where agitators, tanks, and heating equipment are available, instant properties play a minor part.

d. According to its intended use the reconstituted product should meet specific requirements. If the use is beverage milk, the absence of a cooked flavor is of importance. If the powder is to be used for cheese making, the milk should have good clotting properties. If used to make recombined evaporated milk, a satisfactory heat stability is necessary. So there are several widely divergent requirements that cannot be reconciled in one powder. For instance, it is not possible to make whole milk powder that has no cooked flavor and at the same time develops no oxidized flavor during storage. For the first only a mild heat treatment is allowed, whereas for the second intensive heating is needed. In the following we will see additional examples of this kind.

e. The product must be free of health hazards, be it toxic substances or pathogenic organisms. Except for the general hygienic measures and checks prevailing in the dairy industry, there are some special aspects.

The approximate composition of some types of powder is given in Table 17.1. There are other kinds of powder, e.g., cream powder, ice cream mix powder, babyfood of various types, calf milk replacers, etc. All of these products have specific requirements. Since the composition of the raw material varies, the composition of powders also varies. Accordingly, one has to tolerate a certain margin. This offers the possibility for (limited) adulteration; for instance, buttermilk powder or whey powder can be added to (skim) milk powder. The presence of a foreign powder can mostly be detected microscopically; but admixture of a small percentage before the drying generally cannot so easily be established. Since whey powder is cheaper than skim milk powder, this kind of ''adulteration'' sometimes occurs.

17.2   MANUFACTURE

Figure 17.1 gives a flow sheet for the manufacture of whole milk powder. Many steps involved will be self-evident. Intense pasteurization is needed to obtain

TABLE 17.1 Approximate Composition (% w/w) of Some Types of Powder

	Powder from			
Constituent	Whole milk	Skim milk	Whey	Sweet cream buttermilk
Fat	26	1	1	5
Lactose	38	51	72	48
Casein	19.5	27	0.6	26
Serum protein	4.8	6.6	8.5	6.2
''Ash''	6.3	8.5	8	8
Lactic acid	—	—	0.2–2	—
Water	2.5	3	3	3

resistance to autoxidation. With the aid of steam compression, vapor from the last stage of the evaporator is often used to supply a part of the heat needed for pasteurization. Then the pasteurizing step and the heating to the required temperature before the evaporation are combined. The concentrate is not always homogenized, especially if atomization is done by means of a nozzle, because the fat globules are effectively disrupted in the nozzle (Section 9.3). Homogenization of highly concentrated milk considerably increases its viscosity (since the transfer of large casein micelles to the fat globules gives the latter such an irregular shape as to increase the effective volume fraction of fat globules plus casein micelles). This increase leads to coarser droplets during atomization, with all drawbacks involved (Section 9.3.5). Consequently, if the concentrated milk is not homogenized, evaporation can be up to a higher dry matter content. Storage (buffering) of the concentrate before atomization is not always applied; it is done partly to overcome differences in capacity between evaporator and drier. The concentrate should not be kept warm for more than a short time to prevent the growth of microorganisms. A refrigerated concentrate generally is too viscous to be atomized readily and it is therefore heated. The latter must be done just prior to atomization because otherwise the viscosity increases again (age thickening); see, for example, Figure 9.9C. The heating can at the same time serve to kill bacteria that may have recontaminated the concentrate (Section 17.3).

Lecithinizing during the drying in the fluid bed is not always applied; it is meant to obtain instant properties (Section 17.5). The so-called gas flushing, essentially displacing air by N_2 or a mixture of N_2 and CO_2, is to remove a considerable part of the oxygen and thereby to improve the stability toward autoxidation (Section 17.7); it can be done once or twice. If it is not done, the powder
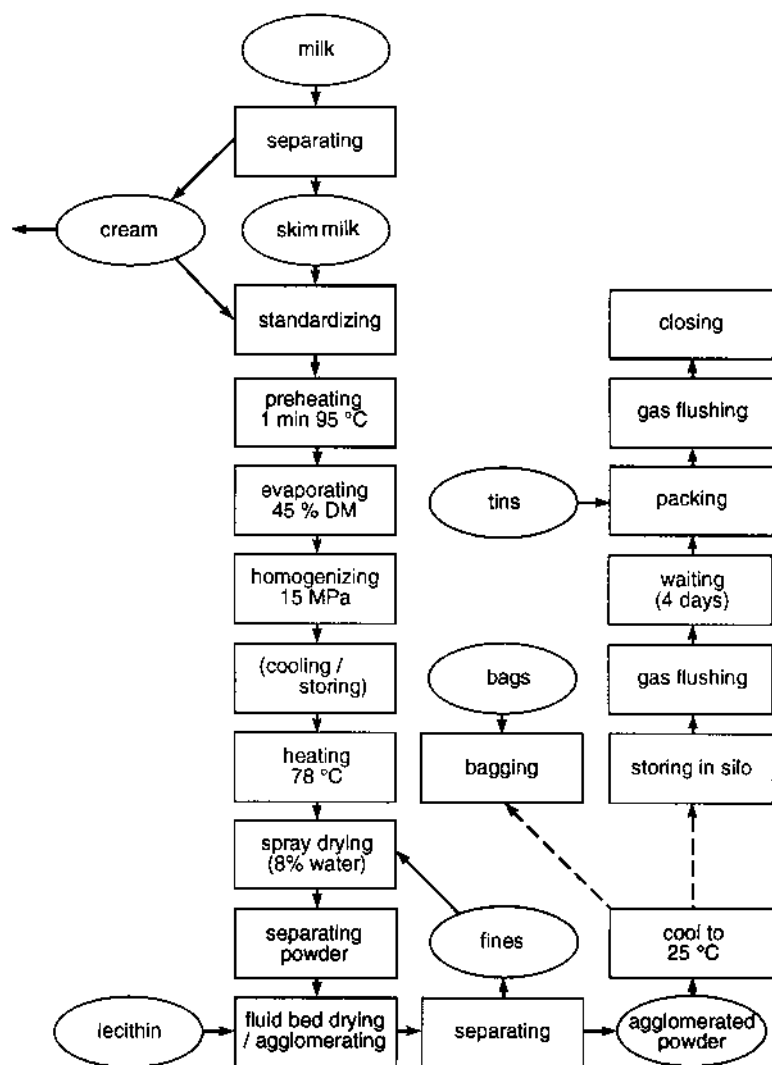
Figure 17.1 Example of the manufacture of whole milk powder.

may be packed into multilayer paper bags with a polyethylene inner layer. Whole milk powder, however, is often packed in tins or in plastic containers to minimize oxygen uptake.

For the production of skim milk powder the pasteurization can be less intense (at least phosphatase-negative), according to the intended application (Section 17.6). Homogenization is omitted, and the milk can be concentrated to a somewhat higher solids content. Nor are lecithinizing and gas flushing carried out. Sometimes vitamin preparations are added, especially vitamin A. This can be achieved by dry mixing afterward, or by emulsifying a concentrated oil solution of vitamin A into a part of the skim milk.

The manufacture of whey powder is largely similar to that of skim milk powder. At first, curd fines should be removed from the whey by filtration or by means of a hydrocyclone, and the whey should be separated. A problem is the processing of sour whey, which causes rapid fouling of the machinery. Sour whey (or skim milk) may be neutralized with alkali.

Whey can be evaporated to more than 60% dry matter, but then crystallization of lactose readily occurs (see Fig. 9.10). An alternative operation is to allow the lactose in the evaporated whey to crystallize as completely as possible, e.g., by keeping it for 3 h at 25°C while stirring. If the dry matter content is over 60%, seeding with lactose crystals is not necessary. Atomization should be with a disk, as a spray nozzle would be blocked. The precrystallized whey powder then obtained has some attractive properties, especially as regards caking (Section 17.7). An additional advantage for the manufacturer is the higher yield: The conventional methods for determining the water content of powders do not remove the bulk of the water of crystallization of α-lactose monohydrate; hence, crystallization of 80% of the lactose yields up to a maximum of 3% ''more'' whey powder. In this way precrystallized skim milk powder also can be made, but then a longer crystallization time and seeding with lactose crystals are needed.

17.3   HYGIENIC ASPECTS

The requirements for the bacteriological quality of milk powder partly depend on its intended use and, in connection with this, also on the manufacturing process. For example, it is of importance as to whether the powder is meant for direct consumption or whether it is subjected to a heat treatment after reconstitution (e.g., for recombined milk). The heat treatment during the manufacture of (skim) milk powder, classified as ''low heat'' (Section 17.6), usually is not more intense than the heat treatment during low pasteurization (say, 72°C for 20 s); consequently, many bacteria may survive the manufacturing process.

The causes for milk powder to be bacteriologically unacceptable or even unsafe can be of three kinds:

a. In the fresh milk bacteria are present that are not killed by the heat treatments to which the milk is subjected before and during drying.
b. Conditions during the various process steps until the product is dry do allow growth of some species.
c. During manufacture, incidental contamination may occur. The level of contamination is generally low and remains low if the bacteria involved cannot grow.

In establishing the bacteriological quality of the powder, the species of bacteria involved should be considered. Table 17.2 gives an overview that applies for one example of the manufacturing process. Then the cause of the contamination may be deduced, as may the measures that must be taken to improve the quality. The table also indicates some attention points.

17.3.1 Bacteria in the Original Milk

In deep-cooled milk psychrotrophic gram-negative rods can develop during prolonged storage (e.g., *Pseudomonas* spp.). As is well known, these bacteria do not survive even a mild heat treatment. Proteases and lipases formed by these rods may survive and become incorporated into the powder. Prevention of the growth of these bacteria (refrigeration, limiting storage time, thermalization) is discussed in Section 5.3. Contamination and growth during storage of the thermalized milk should be avoided.

Of greater importance are heat-resistant bacteria and bacterial spores. They survive low pasteurization (72°C for 15 s), and most are not killed during evaporation and drying. Due to concentrating, the powder contains about 10 times as many bacteria per gram as the milk immediately after preheating. A more intense pasteurization will kill the heat-resistant streptococci (e.g., *S. faecalis, S. thermophilus*) and in a high-quality medium-heat or high-heat milk powder only bacterial spores and *Microbacterium lacticum* can originate from the original milk.

Among the aerobic and anaerobic spore-forming bacteria, especially *Bacillus cereus* and *Clostridium perfringens*, are important to the powder quality. If the reconstituted milk is to be used for cheese making, a very low count of gas-forming anaerobic spore-forming bacteria *(C. tyrobutyricum* and *C. butyricum)* may be essential. All of these bacteria are likely to originate mainly from contamination during milking (dung, soil). A low count of anaerobic spore-forming bacteria points to a good-quality silage (silage being the source of most of the clostridia: feed → cow → dung → milk). But the pathogenic *C. perfringens* usually does not originate from the silage, though from the dung. Hence, a low count of anaerobic spore-forming bacteria need not be an indication of the absence of *C. perfringens*. Likewise, the total count of aerobic spore-forming bacteria is not always an indication for the spore number of *B. cereus*. Usually, the total count is higher during winter, but the count of *B. cereus* may be highest in summer

TABLE 17.2 Example, For a Given Process Scheme, of the Events Occurring With Important Bacteria and Bacterial Enzymes During Manufacture of Dried (Skim) Milk[a]

				Pasteurization			Evaporation		Balance			
Process step	Raw milk	Thermal-izing	Storage	Regen.[b]	Low	High	st. 1–3	st. 4–6	tank	Drying	Packing	Storage
Temp. (°C)	5	65	6	6–65	72	95	70–58	58–42	40	40–100	25	amb.
Duration (min, days)	3 d	¼ m	2 d	1 m	¼ m	¼ m	6 m	8 m	2 m	4 m	3 d	
Dry matter content (%)	9	9	9	9	9	9	9–30	30–50	50	50–96	96	96
Psychrotrophs	G*	K	C, (G)*	(S)	K	K						
Heat-resistant enzymes	F	S	(A, F)	S	S	S		(G)		S	S	P
Staphylococcus spp.	C	(S)	S	(S)	K	K	(C)	(G)	C*, G	(S)	(C)*	P
Enterobacteria	C	(S)	S	(S)	K	K			C*	(S)	C*	P
Salmonella spp.	C	(S)	S	(S)	K	K			C*	(S)	C*	P
Streptococcus thermophilus	(C)	G*	S	G*	S	K	S			(S)	S	P
S. faecalis, S. faecium	C	S	S		S	K	S	G	G*	(S, C)	S	P
Bacillus cereus	C	S, (C)	C	C*	S	(S)	(C)	(C)	(C)*	S	S	P
B. stearothermophilus	(C)	C	S	C	S	S	G	G	G*	S	S	P
Clostridium spp.	C*	S	S		S	S				S	S	P

A, active; C, contamination possible; F, formed; G, growth; K, killed; P, can be present; S, survive; (), partly, occasionally; *, attention point.

[a] Two alternative pasteurization intensities are given.

[b] Regen. = heat regeneration section.

and autumn. This probably is because at higher environmental temperatures *B. cereus* can develop and sporulate in imperfectly cleaned and disinfected machinery, outside the operating periods. Obviously, during the first few hours of processing, slightly higher counts of *B. cereus* are found than later on. To kill bacterial spores, heat treatment at 90–110°C for 10–20 s is insufficient; a UHT treatment should be applied. The D value is about 4 s at 125°C for *B. cereus* as well as for *C. perfringens*, so that heating for 15–20 s at that temperature would cause a sufficient reduction of these spores.

17.3.2   Growth During Manufacture

Temperature and water activity during the successive steps in the manufacture are such that some thermophilic bacteria can readily grow, while they are not or insufficiently killed during drying. The type of bacterium often is characteristic of the cause of the contamination. Some examples will be discussed.

In the regeneration section of pasteurizers and thermalizers (and possibly in the part of the evaporator plant where the milk is heated in counterflow), *S. thermophilus* can develop in particular. The bacterium grows fastest at 45°C but scarcely at temperatures over 50°C. It does not grow in the evaporator because the temperature is too high in the first effects, whereas in the later effects a_w mostly is too low. Since *S. thermophilus* is moderately heat-resistant, it causes increased counts, especially in low-heat milk powder and in whey powder. In medium and high-heat powder the bacterium is killed during manufacture. Determination of the count of *S. thermophilus* in the milk just before the preheater may give a good indication of the fouling of the heating section of the evaporator and of the moment at which it should be cleaned. In some drying plants that employ a wet washer to recover powder fines, the outlet air is brought into contact with a film of milk rather than water, thereby preheating the milk and saving energy; this implies that the preheated milk acquires the wet bulb temperature (about 45°C), which leads to ideal growth conditions for *S. thermophilus*.

The conditions in the second half of the evaporator and in the balance tank are not optimal for *S. thermophilus*. Especially D streptococci (*S. faecium* being an important representative) will start to grow. If the milk is properly preheated and the plant is satisfactorily cleaned and sterilized, it will take a rather long time, however, before substantial counts of *S. faecium* have developed. In actual practice these prerequisites are not always met and in milk powder with a relatively high count, *S. faecium* often is the predominant species.

Likewise, the conditions in the second part of the evaporator and in the balance tank are favorable for growth of *Staphylococcus aureus*. The bacterium generally is killed by pasteurization, and strains of *S. aureus* in milk powder have been shown to have phage characteristics different from the strains in raw milk. Presumably, they originate from direct or indirect human contamination. Heat-

stable enterotoxins can be formed at counts of 10^7–10^8 per ml. The amounts formed may cause the powder to be a health hazard. Although *S. aureus* is not heat-resistant, the conditions during drying appear to be such that complete killing is not achieved. Roughly 10^{-5} to 10^{-1} of the initial count of these bacteria have been found to survive under various practical conditions. This means that *S. aureus* can at least be found in 1 g of fresh powder if its count before the drying was so high that production of enterotoxins could have occurred.

Bacillus stearothermophilus (variant *calidolactis* in particular) can readily grow at higher temperatures. Its growth range is from 45°C to 70°C, with an optimum near 60°C. It can also grow in concentrated milk and therefore throughout the equipment between preheater and drier. Moreover, the bacterium can form spores under these conditions, which further limits its killing during drying. Obviously, some growth of *B. stearothermophilus* will always occur during manufacture, even in a well-cleaned and disinfected plant. However, under normal conditions this will not cause problems.

With regard to the growth of bacteria during the manufacturing process, a number of measures will have to be taken to prevent the equipment from becoming a kind of fermentor for bacteria. Special attention should be paid to the temperature, to the time for which the product stays in the equipment, and to matching of the capacities of the various parts of the plant. Multiple-effect falling film evaporators are more satisfactory in this respect than are flash evaporators. (If it is desirable to achieve as high a concentration factor as possible, it should be considered that the viscosity of the product in the last effect will be quite high, hence also its holdup time.) The balance tanks need special attention; it is recommended that the volume of the concentrate balance tank be kept as small as possible. Mostly, there are two tanks, making possible a change every 2 h or so, and the cleaning of one while the other is in use. Contamination of the product flow from outside should be prevented, with particular reference to *S. aureus*.

The concentrated milk may be pasteurized just before it enters the drier, and this is applied increasingly. It is especially successful with regard to *S. faecalis* and *S. faecium*, provided that the temperature applied is high enough. Heating for 45 s at 72°C has little effect; 45 s at 78°C causes a considerable reduction. Due to the decrease in water activity the *D* and *Z* values have slightly increased (see Table 6.5).

17.3.3 Incidental Contamination

A distinction can be made between contamination before the drying (wet part) and during or after the drying (dry part). Bacteria involved in these types of contamination generally do not grow during the process and contribute little to the count of the powder.

Contamination after preheating and before drying can readily occur if the

equipment has been insufficiently cleaned. This is only important if the bacteria involved can survive the drying (and the pasteurization before drying, if carried out). In spite of the high inlet and outlet temperatures of the air, the concentrate droplets usually will not attain a high temperature (Section 9.3); moreover, the heat resistance of the bacteria increases markedly with the dry matter content. About 70% of *S. faecalis* and *S. faecium* survive during drying, whereas survival of *S. aureus* varies widely. About 10^{-4} to 10^{-5} of the initial count of *Salmonella* spp. and *E. coli* will survive. Based on these facts, and due to the relatively low level of contamination, the powder may be expected to contain no appreciable counts of enterobacteriaceae, immediately after the drier. This is confirmed in actual practice.

Contamination of the powder can occur at many places—in the spray drier, during fluid bed drying, and during packing. The species of contaminating bacteria can vary widely, but it usually concerns species that can grow in wet remnants of milk powder in the drier or in the surroundings of the manufacturing line. Contamination via (in)direct human contacts should also be considered (e.g., *S. aureus*). Bacteria can easily survive in dry powder, and undesirable bacteria can start to grow if the water content increases to over 20%. The supply of cooling air into the drier and into the fluid bed drier can be a source of direct contamination. It may also be responsible for indirect contamination because it gives, at certain sites, better conditions for survival and growth of bacteria in remnants of not fully dried powder. Special precautions are needed if the drier and its accessories have been wet-cleaned. To restrict such incidental contamination, the plant and its surroundings should be rigorously freed of remnants of (wet) powder.

Among the kinds of bacteria found in this type of contamination enterobacteriaceae are of special interest. It generally concerns coliforms; this is possibly caused by lactose being the only carbon source in this environment. Nevertheless, the use of coliforms as an indicator organism is of restricted value for milk powder: If coliforms are absent, salmonellae or other pathogenic bacteria may still be present.

17.3.4 Sampling and Checking

Bacteria originating from contamination or growth prior to drying will usually be homogeneously distributed throughout the powder and cause no problems with sampling. This is different for bacteria originating from incidental contamination of the powder, which may be distributed quite inhomogeneously. It is not possible to devise sampling schemes that guarantee detection of incidental contamination. To ensure that a product is bacteriologically safe, not only the powder should be sampled; indeed, samples must also be taken at sites that are potential sources of contamination. Table 17.2 indicates examples of such attention points.

17.4 PHYSICAL PROPERTIES

A *milk powder particle* generally consists of a continuous mass of amorphous lactose and other low-molar mass components in which fat globules, casein micelles, and serum protein molecules are embedded. The lactose generally remains amorphous, the time available for its crystallization being too short (see also Section 2.1). If, however, precrystallization has taken place, large lactose crystals may be present (some tens of micrometers in size). If lactose has been allowed to crystallize afterward due to water absorption (Section 17.7), its crystals are generally small (about 1 μm). Most fat globules are less than 2 μm, but a small proportion of the fat (e.g., 2% of it) is to be found as a thin layer on parts of the surface of the powder particles. The formation of vacuoles in the powder particles is discussed in Section 9.3. The vacuole volume v of most powders amounts to 50–400 cm$^3 \cdot$ kg^{-1}. An impression of the shape of the powder particles is given in Figure 9.21. When precrystallized whey powder is examined microscopically, most of the particles look more like lactose crystals of the tomahawk shape to which other material adheres.

Examples of *particle size distributions* of milk powder are given in Figure 9.14. Usually, d_{vs} is between 20 and 60 μm and the distribution is relatively wide: c_s amounts to 0.4–0.7. The particles in agglomerated powder are much larger, up to 1 mm, and are irregular in shape. Such a powder usually contains very few separate small particles (less than, say, 10 μm). Within one sample of nonagglomerated powder the larger particles have on average a higher vacuole volume, partly because a drying droplet shrinks more strongly if it contains no vacuoles.

The *density* of a powder may be defined in various ways. The density of the particle material, i.e., excluding the vacuoles, is called the *true density* ρ_t. We have approximately (see also Section 1.2.1):

$$\text{Dried whole milk} \quad \rho_t = 1300 \text{ kg} \cdot \text{m}^{-3}$$

$$\text{Dried skim milk} \quad \rho_t = 1480 \text{ kg} \cdot \text{m}^{-3}$$

$$\text{Dried whey} \quad \rho_t = 1560 \text{ kg} \cdot \text{m}^{-3}$$

The *particle density* ρ_p includes the vacuoles and is therefore given by (v in m$^3 \cdot$ kg^{-1}):

$$\rho_p = \frac{\rho_t}{1 + v\rho_t} \tag{17.1}$$

In skim milk powder ρ_p usually is 900–1400 kg $\cdot$ m^{-3}. The *bulk density* or packing density of the whole powder, ρ_b, is given by

$$\rho_b = \rho_p (1 - \epsilon) = \rho_t \frac{1 - \epsilon}{1 + v\rho_t} \tag{17.2}$$

where ϵ is the void volume fraction or porosity, i.e., the volume fraction of voids (pores) between the particles. Generally, $\epsilon = 0.4$–0.75, but it depends on the way of handling the powder. It decreases considerably when a lightly bulked mass of powder is set by means of tapping or shaking, e.g., from 0.70 to 0.45 for whole milk powder and from 0.55 to 0.40 for skim milk powder. ϵ depends also on other factors (see below). All in all, ρ_b can widely vary, for instance, from 300 to 800 kg · m^{-3}.

     Free-flowingness refers to the ability of a powder to be poured. The property may be determined by pouring out a heap of powder under standardized conditions and measuring its angle of repose α. This is illustrated in Figure 17.2. The more readily the powder flowed, the smaller the angle; thus the more free-flowing it is. The cot α therefore is an appropriate measure of free-flowingness. If the powder is not free-flowing it may be called sticky, i.e., the particles do not readily tumble over each other. Consequently, the porosity ϵ will, in turn, be high. Examples are given in Table 17.3. It turns out that skim milk powder is more free-flowing than whole milk powder and that agglomeration decreases free-flowingness, at least for skim milk powder. With increasing water content of the powder the free-flowingness at first often increases slightly, but it considerably decreases at still higher water contents (e.g., >5%). Usually, the free-flowingness is slightly better at lower temperature. It can be considerably improved
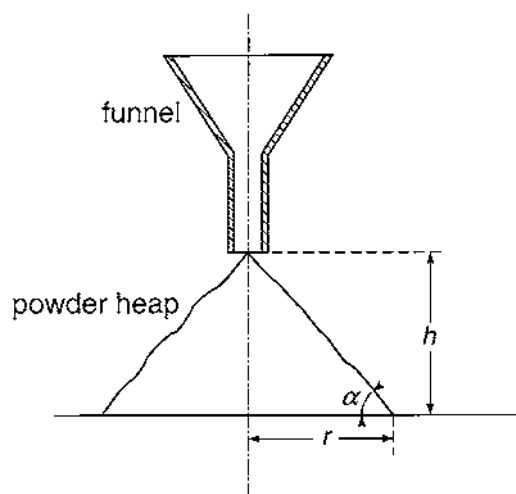
FIGURE 17.2 Determination of the angle of repose α of a powder heap, formed after pouring the powder through a funnel stem. Free-flowingness can be expressed as cot $\alpha = r/h$.

TABLE 17.3 Examples of the Cotangent of the Angle of Repose α (as a Measure for the Free-Flowingness) and the Porosity ε (Lightly Bulked) of Some Types of Powder

Powder	Additive	cot α	ε
Whole milk	—	0.45	0.74
Skim milk	—	0.97	0.57
Instant skim milk	—	0.75	0.73
Whole milk	2% $Ca_3(PO_4)_2$	1.19	0.56
Skim milk	Same	1.28	0.54
Instant skim milk	Same	0.93	0.63
Whole milk	0.5% SiO_2	1.23	0.51

by adding some inert very fine powders like SiO_2, Na-Al-silicate, or $Ca_3(PO_4)_2$ (see Table 17.3). Such additions are indeed applied in milk powder for coffee machines.

17.5 EASE OF DISPERSING; INSTANT POWDER

An important property of milk powder is its ease of dissolution in water or, more precisely, of dispersing it. With hot water and a high-speed agitator, dissolution causes few problems, but dissolving the powder in cold water under household conditions may be far from easy. Instant powder disperses rapidly in cold water with gentle stirring. The dispersibility is not related to the solubility of the powder, but to the rapid penetration of water in a mass of powder. Due to this penetration the powder particles disperse separately in the water, where they can subsequently dissolve.

The instant properties depend on the phenomena occurring when a quantity of powder is brought into or upon water:

a. The powder should be wettable by the water. Consider two powder particles close together on a water surface (Fig. 17.3). The wetting depends on the contact angle (θ, as measured in the aqueous phase) of the system consisting of dried milk, water, and air. The contact angle depends on the three interfacial tensions: solid–water, solid–air, air–water. For a hydrophobic solid θ is large, for a hydrophilic solid it is small. If θ is less than 90°, the particles are wetted. For dried skim milk θ is about 20°, for dried milk about 50°. This would imply that water is always sucked into the pores between the particles by capillary forces: If the water surface is curved, the capillary force acts in the
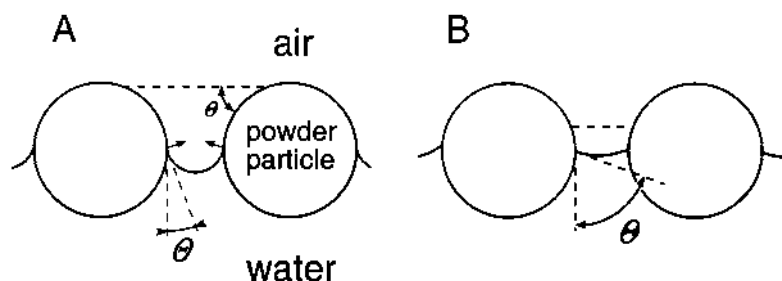
FIGURE 17.3   Capillary rise of water between two powder particles. θ = angle of contact. The broken line gives the situation where (for cylinders) the water surface is flat. (A) θ = 20°; (B) θ = 70°. Highly schematic.

direction of the concave side, i.e., upward in the case of Figure 17.3. In skim milk powder this is indeed the case in practice. But Figure 17.3 shows that the maximum height to which the water can rise between two powder particles, which is until a flat water surface is reached, is greater for a smaller θ. In actual practice, the situation is more complicated (three-, not two-, dimensional situation; surface of the powder particles is not smooth), but in a qualitative sense similar phenomena will occur; during wetting of a powder mass there is an effective contact angle θ_{eff}, which is greater than the contact angle on a plane surface. In whole milk powder θ_{eff} may be greater than 90°, especially if the fat is partly solid; the consequence is that water does not penetrate the powder mass or does so only locally. The remedy is to cover the powder particles with a thin layer of lecithin, thereby considerably decreasing θ_{eff}.

b.   The speed of penetration of the liquid into the powder is slower for a larger θ_{eff}. The speed is about proportional to the average diameter of the pores between the particles and inversely proportional to the liquid viscosity. The smaller the powder particles and the wider the spread in size (allowing smaller particles to fill the voids between the larger ones), the narrower the pores. In normal skim milk powder the pores are too narrow to permit rapid penetration of water.

c.   Problem b above is aggravated by the pores becoming narrower during wetting. This is because the capillary action of the penetrating water pulls partly wetted particles closer to each other, especially if θ is small (see arrows in Fig. 17.3A). Due to this capillary contraction the powder mass may decrease by 30% to 50% in volume upon wetting.

d.   Penetration of the aqueous solution is also slowed down by dissolution

    of powder constituents in the water, thereby increasing liquid viscosity. Lactose, the main constituent, is present in an amorphous state, in fact an extremely concentrated solution; hence, it dissolves quickly (one may even say that it becomes diluted) to form a highly viscous solution.

e. Due to phenomena b–d, the penetration of water will soon stop. Lumps of powder are formed, which are dry inside and have a highly viscous outer layer of highly concentrated milk. Such lumps dissolve very sluggishly.

f. Other properties of the powder may slightly affect the dispersibility, e.g., the force needed to pull adjoining particles apart; or the particle density, which determines whether the particles will sink. Generally, these aspects are not decisive.

To give the powder instant properties, it thus should be allowed to agglomerate so that at most a few fine particles are left; because of the agglomeration, the pores through which the water primarily penetrates are much wider. This means that the agglomerates are readily dispersed; subsequently, they dissolve, which may take a minute or so. It is also important that the agglomerates be strong enough to avoid their easy disintegration if the powder is subjected to external forces, as during packing and shaking. These properties depend on the conditions in the fluid bed drier (Section 9.3). In addition, whole milk powder should be lecithinized to ensure a small contact angle.

17.6 INFLUENCE OF PROCESS VARIABLES ON PRODUCT PROPERTIES

Several properties and quality features of milk powder can be strongly affected by the means of manufacture. Some aspects have been discussed above (see also Section 9.3).

17.6.1 Flavor

Milk powder often has a cooked flavor, which results from the flavor compounds formed during preheating and possibly during evaporation. During drying, conditions are mostly not such that off-flavors are induced. On the contrary, a considerable part of the volatile sulfhydryl compounds (especially H_2S) is removed. Obviously, a cooked flavor in milk powder mainly results from methyl ketones and lactones formed by heating of the fat (they thus are almost absent in skim milk powder) and from Maillard products. Flavor changes during storage are discussed in Section 17.7.

17.6.2 WPN Index

Heat treatment of the original product, concentrate, and/or drying droplets can cause denaturation of serum proteins, though the conditions during drying are

rarely such as to cause extensive heat denaturation (Section 9.3). The extent of denaturation is an important quality mark in connection with the use of milk powder. If the dissolved powder is to be used for cheese making, practically no serum protein should have been denatured in view of the rennetability; in infant formulas, on the other hand, the rennetability should be poor.

The extent of the denaturation of serum protein can be used as a measure for the heating intensity applied. This also holds where denaturation by itself may be of no importance but other changes, associated with intense heat treatment, are. An example is the flavor of powder to be used in beverage milk, which requires a mild heat treatment. A good stability against heat coagulation in the manufacture of recombined evaporated milk, or a high viscosity of the final product in the manufacture of yogurt from reconstituted milk, requires intensive heat treatment. The latter is also desired if the powder is used in milk chocolate; presumably, Maillard products contribute to its flavor.

The whey protein nitrogen (WPN) index is generally used to classify milk powders according to the intensity of the heat treatment(s) applied during manufacture. To that end, the amount of denaturable protein left in the reconstituted product is determined, usually by making acid whey and determining the quantity of protein that precipitates on heating the whey. This can be done by Kjeldahl analysis of protein nitrogen or by means of a much easier turbidity test that is calibrated on the Kjeldahl method. The result is expressed as the quantity of undenatured serum protein per g skim milk powder. The classification is as follows:

WPN $\geq$ 6 mg N per g: ''low heat''
6 > WPN > 1.5 mg N per g: ''medium heat''
WPN $\leq$ 1.5 mg N per g: ''high heat''

The validity of the test is moderate. Due to the natural variation in the amount of denaturable protein in raw milk (recalculated as WPN index 6.5–10) and to the limited reproducibility of the test, a substantial portion of the serum protein (up to 40%) may have been denatured in a powder classified as ''low heat,'' and in a ''high heat'' powder up to 23% may be undenatured. Of course, the average validity is far better.

If one wants to make ''low-heat'' powder, one should apply low pasteurization (e.g., 15 s at 72°C); begin the evaporating process not above 70°C (and preferably somewhat lower); evaporate the milk not too far; keep the concentrate temperature below 60°C; cool down the concentrate if it is to be kept for a long time; and maintain the outlet temperature during spray drying at a low level. Moreover, the mixing of air and droplets in the drying chamber should be such that no local overheating of the drying droplets can occur. If one wants to make ''high-heat'' powder, the milk should be intensely heated, e.g., 5 min at 90°C or 1 min at 120°C; often even higher temperatures are used. A more intense

preheating causes a higher viscosity of the concentrate at the same dry matter content (Fig. 9.9), with all of its consequences (Section 9.3.5).

17.6.3 Insolubility

Insolubility can be determined in various ways. In all tests, powder is dissolved under standardized conditions (concentration, temperature, and duration and intensity of stirring), then the fraction that has not been dissolved is determined (e.g., volumetrically after centrifugation or by determination of dry matter). Often one refers to this as a ''solubility index,'' but this is a confusing expression because it concerns an insolubility figure or insolubility index. The insoluble fraction—essentially the material that sediments during centrifugation—will predominantly consist of protein. In whole milk powder, flocks of coagulated protein with entrapped milk fat globules (the so-called flecks) may float to the surface; the quantity involved usually is more than the sediment. Hence, the insolubility found closely depends on the method used. Some examples are given in Table 17.4.

The insolubilization of a fraction of the milk powder is related to heat coagulation (Section 6.2.4). Consequently, the extent to which it occurs greatly depends on the time during which the drying material is at high temperature and on the degree of concentration during drying. The determinant factors are dis-

TABLE 17.4 Some Examples of the Insolubility of Whole Milk Powder, Determined With Different Methods

| Powder | | | CCF | |
sample	ADMI	ZKB	x	y
1	0.02	0.04	0.2	0
2	0.02	0.04	2.2	0.1
3	0.04	0.07	2.1	0.1
4	0.08	0.04	1.3	0.1
5	0.10	0.04	2.6	0.3
6	0.16	0.12	1.6	0.2
7	1.1	—	2.8	0.5
8	3.4	—	0.9	0.8

ADMI: ml sediment per 50 ml of reconstituted milk; dissolving at 24°C, intensive stirring; centrifuging.
ZKB: as ADMI, but at 50°C and less intensive stirring.
CCF: in g per 30 g powder; dissolving at 20°C, gentle stirring; centrifuging; x is excess of dry matter in top layer, y same in bottom layer.

cussed in Section 9.3 and the influence of some product and process variables is summarized in Figures 9.22B and 9.23. Furthermore, the preheating has an effect: high preheating → higher viscosity → larger droplets on atomization → more intense heat treatment during drying → increased insolubility. But evaporation to a certain viscosity would imply a lower degree of concentration for highly preheated milk, so that heat coagulation and insolubilization during drying would be less. Finally, homogenization of the concentrate may increase the insolubility (specially the term x in the CCF test; see Table 17.4), but this may hardly be noticeable because considerable homogenization occurs during evaporation and atomization. (The insolubility of whole milk powder is indeed increased more readily than that of skim milk powder.) Homogenization of the concentrate may also lead to greater insolubility if it considerably raises its viscosity (thus at a high degree of concentration), increasing the droplet size and thereby the drying time.

17.6.4   Specific Volume

Specific packed or bulk volume is equal to $1/\rho_b$ [Eq. (17.2)]. Sometimes a distinction is made between specific bulk volume (if the powder is lightly bulked) and specific packed volume (if the powder is allowed to set, e.g., by tapping). For determination of the latter, a tapping apparatus may be used, which repeatedly brings a graduated cylinder with powder to fall on a solid base. Obviously, the packed volume is of great importance in connection with the mass of powder that can be stored in a certain package. The volume should not be greatly reducible by tapping because otherwise a can, being full of powder when filling, may appear to be partly empty later on when used.

Equation (17.2) shows that the variables determining ρ_b are vacuole volume (v) and porosity (ϵ). The process variables affecting v are summarized in Figure 9.15. Generally, factors causing a higher viscosity of the concentrate during atomization, and a lower average drying temperature will lead to a lower v and thus to a higher ρ_b. The drying temperature, however, may also affect ϵ. Other factors affect ϵ (see, e.g., Table 17.3), and not all of those are easy to explain. In general, ϵ will be higher if the powder particles are more irregular in shape and differ less in size. Accordingly, agglomeration and removal of small powder particles clearly raise ϵ, hence decrease ρ_b. ϵ may decrease slightly with increasing water content of the powder; presumably, it causes the particle surface to become smoother. Precrystallization of lactose makes the particles more angular and raises ϵ slightly.

Obviously, ρ_b increases due to shaking, tapping, or vibrating. In general, the effect produced is reversible. But in agglomerated powder the agglomerates can be disrupted; consequently ρ_b is increased irreversibly and the instant properties are impaired.

17.6.5 Free Flowingness

Free-flowingness is discussed in Section 17.4. It is strongly correlated with the above-mentioned porosity: the higher ϵ, the poorer the free-flowingness.

17.6.6 Free Fat Content

The term ''free fat content'' is, properly speaking, incorrect because it suggests that some fat is entrapped in the powder particles without a surrounding membrane. So-called free fat is determined by extraction with an organic solvent. In fact, the fat is then extracted from all fat globules that are in contact with the surface of the particle, or with a vacuole, with pores or cracks, etc. This is illustrated in Figure 17.4. Obviously, the amount of extractable fat will be higher as the powder particles are smaller, a greater number of vacuoles have been entrapped, and the drying has been performed at a higher temperature (giving more cracks) (see also Figs. 9.15B and 9.22C). Increasing the water content of the powder decreases the amount of extractable fat since cracks are closed due to swelling. The methods of extraction are, moreover, quite empirical and the result greatly depends on small variations in the analytical procedure.

The content of extractable fat is often used as a quality mark, but this makes
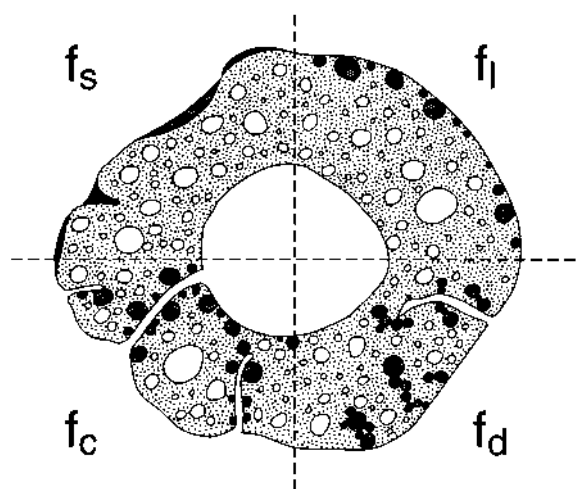
FIGURE 17.4 Diagrammatic model of a milk powder particle, indicating which part of the fat (hatched) can be extracted. In each quadrant another type of extractable fat is indicated. From T.J. Buma, *Neth. Milk Dairy J.* **25**(1971)159–174.

little sense because it hardly correlates with other product properties (except sometimes with the rate at which vacuoles in the powder particles become filled with air). Quadrant f_s in Figure 17.4 indicates that part of the fat is located on the surface of the powder particles. Such fat may be estimated by means of a very brief (say, 3 s) extraction at low temperature. It correlates more or less with the contact angle for wetting of the powder and hence with the dispersibility (more surface fat → poorer wettability). It would be far better to estimate the fraction of the particles' surface that is covered with fat; an impression can be obtained by microscopy of powder stained with an oil-soluble dye.

The amount of extractable fat can be reduced by intensive homogenization. Only in milk powder with less than 20% fat does this affect the dispersibility, and then only slightly.

17.6.7 Dispersibility

Dispersibility is discussed in Section 17.5 together with the factors determining it. The influence of process variables can easily be deduced.

17.6.8 Stability

Changes in properties during storage are discussed in Section 17.7.

17.6.9 Conclusion

Figure 17.5 summarizes the influence of some process variables on six product properties. Such a summary is inevitably an oversimplification because the relationship between two parameters may depend on other variables. For instance, the curves for the WPN index (except for the extreme left-hand one) have been drawn for a mild preheating; otherwise they would exist at a much lower level. Moreover, it is mostly not possible to vary only one process variable. Except in the upper row, the conditions (usually the concentrate supply in the drying chamber) supposedly have been adjusted in such a way that the water content of the powder remains constant. An exception is the outlet temperature: If this is very low the water content of the powder will inevitably be high, and additional drying should then follow, e.g., in a fluid bed. Note that such a second-stage drying provides more possibilities to make powder with various desirable characteristics.

17.7 DETERIORATION

The most important parameter determining the rate of undesirable changes in milk powder is the water content. When comparing different types of powder, it is probably easiest to consider water activity (a_w), as, for instance, in Figure 9.4. Examples of the relation between water activity and water content are shown
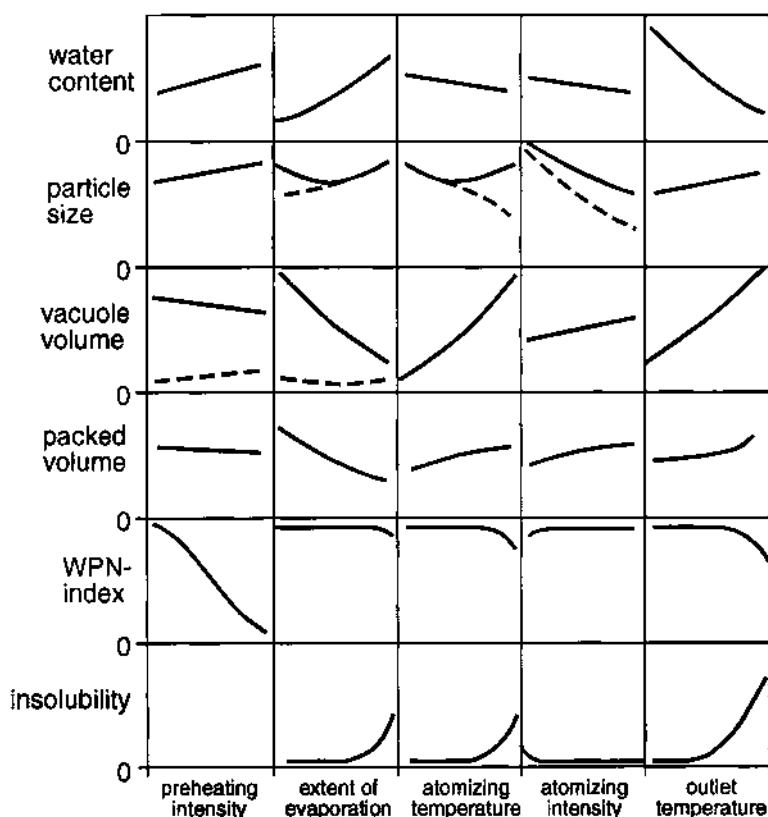
FIGURE 17.5 The influence of the intensity of preheating of the milk, of the extent of evaporation of the concentrate, of the temperature and the intensity (disk speed, pressure) of atomization, and of the outlet temperature of the drying air on some properties of the spray-dried milk. Very approximate. (---) For conditions at which vacuoles develop hardly or not at all.

in Figures 9.3 and 9.16. The relationship depends on the composition of the product; some examples are given in Table 17.5. The higher a_w of whole milk powder as compared to skim milk powder at the same water content is caused by the fat not affecting a_w. Whey powder has an a_w slightly different from that of skim milk powder because in a dry product the soluble constituents (especially sugar and salts) decrease a_w somewhat less than casein. This only holds, however, as long as all lactose is amorphous, which often does not apply to whey powder. The data in Table 17.5 show that a_w is considerably reduced if lactose crystallizes without the powder absorbing water (compare rows 1 and 5), at least at a_w less

TABLE 17.5 Approximate Water Activity of Various Kinds of Spray Powder as a Function of the Water Content and of Some Other Variables

Powder made of	Temp. (°C)	State of lactose	Water content (% w/w)			
			2	3	4	5
1 Skim milk	20	Amorphous	0.07	0.13	0.19	0.26
2 Whole milk	20	Amorphous	0.11	0.20	0.30	0.41
3 Whey	20	Amorphous	0.09	0.15	0.20	0.26
4 Skim milk	50	Amorphous	0.15	0.24	0.33	0.42
5 Skim milk	20	(crystalline)[a]	0.02	0.04	0.06	0.12
6 Skim milk	20	crystalline[b]	0.09	0.16	0.25	0.38

[a] Water content of powder includes water of crystallization; the lactose thus is crystallized insofar as sufficient water is present for the crystallization.
[b] Water content of powder does not include water of crystallization.

than about 0.5 (see Fig. 9.3b). Crystalline lactose thus binds water very strongly and that is also why the usual methods to determine the water content do not include the bulk of the water of crystallization. If the water content excluding the water of crystallization is taken as a basis, then a_w is even higher for the powder with crystallized lactose; compare rows 1 and 6 in Table 17.5.

It thus is advisable to make milk powder sufficiently dry and to keep it sufficiently dry. If it is not hermetically sealed from the outside air, it will attract water in most climates. The higher the temperature, the higher the water activity; see Table 17.5 (compare rows 1 and 4) and Figure 9.17. Because several reactions are faster at a higher a_w, this implies that a temperature increase often causes an extra acceleration of deterioration.

Microbial and *enzymic* deterioration are rare in milk powder. For microbial deterioration to occur, a_w should increase to over 0.6 (and for the majority of microorganisms much higher); such a high a_w is only reached if the powder is exposed to fairly moist air. Deterioration then is often caused by molds. Enzymatic hydrolysis of fat has been observed at $a_w \geq 0.1$, be it extremely slow. Accordingly, whole milk powder must be free of lipase. Milk lipase will always be inactivated by the intense pasteurization of the milk as applied in the manufacture of whole milk powder. This is by no means ensured, however, for bacterial lipases. Hence, not too many lipase-forming bacteria should occur in the milk. Proteolysis in milk powder appears highly improbable and it has never been reported.

Of course, enzymic deterioration of liquid products made from the milk powder can occur if enzymes are present before the drying, since drying usually does not cause substantial inactivation of enzymes (see Section 9.3.5).

Caking. What may be noticed first when milk powder or whey powder

absorb water from the air is that lumps are formed; eventually, the whole mass of powder turns into a solid mass (cake). Crystallization of lactose is responsible, as it causes the powder particles, largely consisting of lactose, to grow together (to sinter). Since water is needed for crystallization of α-lactose, caking does not occur at low a_w, say, below 0.4. At a higher temperature crystallization can occur far more readily, a_w being higher; moreover, the viscosity of the highly concentrated lactose solution (essentially the continuous phase of the powder particles) is lower, causing nucleation, hence crystallization, to be faster.

The susceptibility to caking, especially high in whey powder, is considerably reduced if most of the lactose is crystallized before the drying (in the concentrate). Such precrystallized powder is usually called ''nonhygroscopic,'' which may be a misnomer because the powder concerned does not attract less water (this is determined by its a_w in relation to that of the air), but the effects differ.

Maillard reactions increase considerably with water content (see Figs. 9.4 and 9.11) and with temperature. They lead to browning and to an off-flavor. The gluey flavor that always develops during storage of dry milk products with a too-high water content is usually ascribed to Maillard reactions; the main component appears to be *o*-aminoacetophenone. If extensive Maillard reactions occur, this is always accompanied by insolubilization of the protein. Accordingly, the insolubility index increases if milk powder is stored for long at a high water content and temperature; at a normal water content the ADMI number (see Table 17.4) may increase to 0.5 in 3 years.

Autoxidation of the fat and the ensuing tallowy off-flavor pose a difficult problem when storing whole milk powder. The rate of autoxidation strongly increases with decreasing a_w (see Fig. 9.4); however, to prevent other types of deterioration (especially Maillard reactions) a_w should be as low as possible. The effective Q_{10} of the autoxidation reaction in milk powder is relatively low (about 1.5) since a higher temperature also causes higher a_w.

To keep the autoxidation within reasonable limits for a long time, a number of measures should be taken (see also Section 2.3):

a. Intense heat treatment of the milk to form antioxidants (see Fig. 17.6). The problem is, of course, that the heat treatment also causes a distinct cooked flavor.
b. Adjusting the water content of the powder to as high as possible without causing Maillard reactions to occur too fast; the most suitable water content is generally 2.5% to 3%.
c. Removing oxygen as effectively as possible (by gas flushing; see Fig. 17.6). A problem is that the vacuoles in the powder particles contain some air, hence O_2. Either the powder should contain hardly any vacuoles or the gas flushing should be repeated after a few days. Equilibrating the gas inside and outside the vacuoles by diffusion takes several
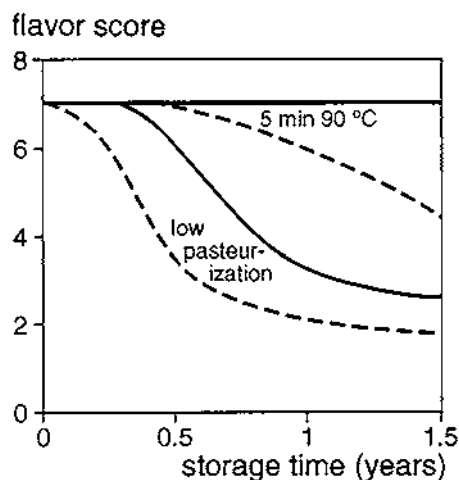
flavor score

FIGURE 17.6 Influence of preheating and of gas flushing, whether (——) or not (---), of whole milk powder on its flavor score (scale 0–8) during storage at room temperature under exclusion of air. After E.A. Vos and J.J. Mol, NIZO-Mededelingen M12 (1979).

    days in whole milk powder (several weeks in skim milk powder). Equilibration is faster if the powder particles have a greater number of cracks (see Section 17.6).

d. Packing the powder in such a way that air and light are kept out. Generally, this implies packing in cans.

e. Rigorous measures against contamination of the milk with copper.

f. Intensive homogenization of the concentrate.

A so-called toffee flavor can develop during storage of whole milk powder with a high water content at high temperature. It can be ascribed to formation of δ-decalactone and related components in the fat. The compounds involved are not formed by oxidation.

Loss of nutritive value during storage primarily concerns loss of available lysine due to Maillard reactions. Storage at 20°C at a normal water content does not cause an appreciable loss; at 30°C a loss of 12% after storing for 3 years has been reported. Extensive Maillard reactions cause a decrease in protein digestibility and formation of weak mutagens.

Extensive autoxidation results in formation of reaction products between hydroperoxides and amino acid residues (this partly gives methionine sulfoxide), and between carbonyl compounds and ε-amino groups; this may cause the biological value of the protein to decrease slightly. Of greater concern is the loss of

vitamin A in vitamin-fortified skim milk powder due to its oxidation. This especially occurs if the vitamin preparation is dissolved in oil and then emulsified into the skim milk before atomization. Usually, dry added preparations are stabler. It is, however, very difficult to homogeneously distribute a minute amount of a powder throughout a bulk mass.

17.8   OTHER TYPES OF MILK POWDER

Roller-dried milk looks completely different from spray powder in the microscope. It consists of fair-sized flakes. Due to the intense heat treatment during the drying it has a brownish color, a strong cooked flavor, and the availability of lysine has been considerably reduced, by 20% to 50%.

Freeze-dried milk consists of coarse, irregularly shaped, and very voluminous powder particles, which dissolve readily and completely. However, the fat globules show considerable coalescence, unless intense homogenization has been applied. In most cases, damage due to heat treatment is minimal.

17.9   RECONSTITUTED PRODUCTS

Milk powders can be used to make a variety of liquid milk products. Some common types are the following:

Reconstituted milk is simply made by dissolving whole milk powder in water to obtain a liquid that is similar in composition to whole milk. Likewise, reconstituted skim milk can be made.

Recombined milk is made by dissolving skim milk powder in water, generally at 40–50°C, then adding liquid milk fat (preferably ''anhydrous milk fat'' of good quality; see Section 19.4.1), making a coarse emulsion by vigorous stirring or with a static mixer, and then homogenizing the liquid. This product is similar to homogenized whole milk, except that it lacks most of the material of the natural fat globule membrane, such as phospholipids.

Other recombined milk products are also made. Figure 16.2 gives a manufacturing scheme for recombined evaporated milk.

Filled milk is like recombined milk, except that instead of milk fat a vegetable oil is used to provide the desired fat content.

Toned milk is a mixture of buffaloes' milk and reconstituted skim milk. The high fat content of buffaloes' milk (e.g., 7.5%) is thereby toned down.

SUGGESTED LITERATURE

- See also Chapter 9.
- A general overview on an introductory level is given by:

    M. Carić, *Concentrated and Dried Dairy Products*, VCH, New York, 1994.

- Properties of dried milk products are discussed by:
    S. T. Coulter and R. Jenness, in: W. B. van Arsdell et al., eds., *Food Dehydration*, 2nd ed., Vol. 2, AVI, New York, 1973, pp. 290–346.
- A survey of the bacteriology of spray-dried milk powders is given by:
    J. Stadhouders, G. Hup, and F. Hassing, *Neth. Milk Dairy J. 36*, 1982, 231–260.
- Practical information on recombined milk products is in:
    Recombination of milk and milk products, *Bulletin of the International Dairy Federation*, Document 142, Brussels, 1982.

18

Protein Preparations

18.1 INTRODUCTION

Over the last few decades an increasing number of dried, milk protein–rich products have come on the market. Traditionally, isolated casein was used for the manufacture of synthetic wool and buttons, for sizing paper (i.e., making it smooth and easy to write on), etc. These uses have much diminished. Currently, the main use of various types of casein and of protein preparations isolated from whey is in manufactured foods. The purpose may be:

1. *Increasing the nutritive value*, e.g., by adding the preparations to beverages or cereal products. The high biological value and digestibility of milk protein are essential. Sometimes, partially hydrolyzed proteins, i.e., peptide mixtures, are applied for people who are allergic to certain proteins.
2. *Providing a product with particular physical properties*. Examples are the preparation of stable emulsions (salad dressings, desserts, coffee whiteners) and of foam products (desserts, toppings, meringues) or the prevention of segregation of moisture and fat in meat products.
3. *Displacing more expensive proteins*. Most animal proteins are more expensive than vegetable proteins, but milk proteins can be comparatively cheap. Moreover, isolation of vegetable proteins such that these are sufficiently pure, flavorless, and functional (e.g., soluble) often is expensive. Ever more proteins from whey or protein-rich whey products rather than milk proteins are used in ices, desserts, beverages, calf milk replacers, etc.
4. *Manufacturing novel products*. This has been enhanced by the availability of new technologies, such as spinning and extrusion. Examples

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are cheese-like sandwich spreads and meat substitutes. Alternatively, new methods of manufacture are often tried, e.g., manufacturing a food from fairly pure and durable components, followed by processing.

Price and functional properties of milk protein preparations are paramount for their possible use. In addition, keeping quality, flavor (or, rather, lack of flavor, since pure proteins are virtually flavorless), and dispersibility are of importance.

Various raw materials are used, including skim milk, sweet-cream buttermilk, and whey if not too acid. Whey is a relatively cheap material, and membrane processes, ion exchange, and other techniques have facilitated its use. Usually, the preparations are finally dried. The range of raw materials and manufacturing processes have introduced a wide range of products (Table 18.1). The protein content and the other components vary widely. The proportion of nonprotein nitrogen in total nitrogen (which often is multiplied by 6.38 to obtain a hypothetical protein content) varies considerably, i.e., from 0 to 30%. There are still other variations. The ''ash content'' may include varying amounts of salts resulting from incineration of protein. The water content may greatly depend on the method of determination. By no means can all of the variations in properties be derived from the gross composition. To that end, the method of manufacture and the storage time and storage conditions should also be considered.

An essential problem in the manufacture of protein preparations may be the waste product. Can it still be utilized or discarded or is expensive purification required?

18.2   MANUFACTURING PROCESSES

The properties of milk protein preparations can markedly depend on pretreatment of milk or whey. Heat treatment is required to kill bacteria and to inactivate enzymes. It can cause denaturation; hence decreased solubility of serum proteins (see Fig. 6.4). Most of the denatured serum proteins are associated with the casein if the (skim) milk has been heated. The cream separation efficiency determines the fat content of the preparations. This content depends also on the extent to which the fat globules, because of their disruption or loss of membrane (e.g., due to beating in of air), have been covered with plasma proteins. Such fat globules follow the protein during its separation and can hardly be removed from the protein preparation by the usual purifications. Bacterial spoilage and plasmin activity cause proteolysis. The extent to which whey has been acidified before accumulation of its protein affects composition and properties of the preparations involved. Rennet whey contains caseinomacropeptide; whey from acid coagulation does not. A preparation from non-heat-treated rennet whey may also contain residual rennet.

TABLE 18.1 Overview of Some Milk Protein–Rich Preparations and Their Composition[a]

Product	Method of preparation	Isolated from	Gross composition (%)			
			Crude protein	Carbohydrate	''Ash''	Fat
Acid casein	Acid coagulation	Skim milk	83–95	0.1–1	2.3–3	~ 2
Na-caseinate	Acid + NaOH	Skim milk	81–88	0.1–0.5	~ 4.5	~ 2
Rennet casein	Renneting	Skim milk	79–83	~ 0.1	7–8	~ 1
WP[b] isolate	Ion exchange	Whey	85–92	2–8	1–6	~ 1
WP concentrate	Ultrafiltration	Whey	50–85	8–40	1–6	< 1
WP concentrate	Electrodialysis + lactose crystallization	Whey	27–37	40–60	1–10	~ 4
Whey powder[c]	Spray drying	Whey	~ 11	~ 73	~ 8	~ 1
WP complex	Metaphosphate	Whey	~ 55	~ 13	~ 13	~ 5
WP complex	CMC	Whey	~ 50	~ 20[d]	~ 8	~ 1
WP complex	Fe + polyphosphate	Whey	~ 35	~ 1	~ 54	~ 1
Lactalbumin	Heat + acid[e]	Whey	~ 78	~ 10	~ 5	~ 1
Coprecipitate	Heat + acid[e]	Skim milk	~ 85	~ 1	~ 8	~ 2

[a] The manufacture always includes drying to some 3% to 8% water.
[b] WP = whey protein.
[c] Shown for comparison.
[d] Includes CMC (carboxymethylcellulose).
[e] And/or $CaCl_2$.

18.2.1 Casein

Various kinds of casein are currently being manufactured.

    a. *Rennet casein* is isolated from skim milk by renneting with calf rennet, while stirring at fairly high temperature which causes rapid syneresis. The fine syneresed curd particles are separated centrifugally or by use of a vibrating sieve, washed with water, pressed to remove water, and dried, e.g., in a drum or a belt drier. The product thus is composed of calcium paracaseinate–calcium phosphate with impurities. It is insoluble in water and has a high ash content.

    b. *Acid casein* is made by acidifying (diluted) skim milk, while stirring, with lactic acid, hydrochloric acid (mostly), or sulfuric acid. Casein precipitates at its isoelectric pH. The temperature applied is quite critical. A high temperature causes large lumps that are difficult to dry; a low temperature causes a fine voluminous precipitate that is hard to separate. At low temperature, acid casein gels show little syneresis (see Section 22.2), and the precipitate formed essentially consists of lumps of a gel. The milk is usually acidified at fairly low temperature. After having increased the temperature, the process is continued as described for rennet casein. The preparation can be purified by dissolving it in alkali, reprecipitating it, etc. Acid casein is insoluble in water and is usually poorly soluble in alkaline solutions because persistent clumps are formed.

    c. *Caseinates.* Acid-precipitated casein can be dissolved in alkali, e.g., NaOH, KOH, NH_4OH, $Ca(OH)_2$, $Mg(OH)_2$, and be spray-dried subsequently. Na-caseinate is the most common product. K-caseinate sometimes is preferred for nutritional purposes; Ca-caseinate has somewhat different physicochemical characteristics. These products can be highly soluble in water and be fairly flavorless if the pH during manufacture was never higher than 7.

Several semiindustrial processes to separate casein into fractions have been described. Full fractionation is not easy. Nevertheless, preparations enriched in α_s- or β-casein can be made.

18.2.2 Whey Protein (WP) Concentrates and WP Complexes

Proteins dissolved in whey can be gathered in various ways.

    a. Ultrafiltration (Section 9.4) of whey is a much applied method. It results in separation as well as concentration. Diafiltration (i.e., adding

b. Gel filtration has the drawback that it does not lead to concentration and, moreover, is expensive. Accordingly, it is rarely applied.

c. Protein separation by ion exchange yields a protein that often mainly comprises β-lactoglobulin and α-lactalbumin. The product obtained, called WP isolate, can be very pure, especially if it is also ultrafiltered to concentrate the protein and to remove dissolved constituents.

d. Evaporating whey to crystallize lactose; then the crystals are removed and the concentrate is desalted. Desalting is mostly done by electrodialysis (Section 9.4). Removing the last amounts of salt requires much energy and, alternatively, ion exchange is therefore applied.

e. Most whey proteins can be precipitated at low pH by carboxymethylcellulose or with hexametaphosphate. The protein then is partly electropositive and the precipitating agent negative, so that these two compounds associate. The whey protein complexes formed have poor solubility at low pH (<5) and of course they contain the precipitating agent. The complexes can also be formed at neutral pH by ferric ions plus polyphosphates; such products have poor solubility and a very high ash content (see also Table 18.1).

f. Reverse osmosis, evaporation, and drying are also applied for concentrating whey. There may be several combinations of process steps, depending on the practical possibility, the effect on product properties, the potential to convert various waste products, and the processing costs.

Spray-dried whey protein concentrates can be highly soluble. The part of the protein that does not dissolve due to heat denaturation greatly depends on the pH and the Ca^{2+} activity during heating (Fig. 18.1). Adding Ca salts after heating requires a higher concentration of Ca^{2+} to insolubilize the protein. About half of the protein in whey consists of β-lactoglobulin, and its properties dominate those of the WP concentrates.

Whey protein isolated by ultrafiltration or by complex formation contains little nonprotein N, e.g., 1% to 3% of the N. On the other hand, some 20% to 30% of the nitrogen in demineralized whey obtained after lactose crystallization may consist of NPN.

18.2.3 Lactalbumin

It has long been a practice to heat sour cheese whey to precipitate the protein (cf. Fig. 18.1) and to recover it. Obviously, the precipitate is impure. It is pressed, usually salted, and sometimes matured. This is ''whey cheese,'' e.g., ricotta or Ziger. A similar process can be applied to yield denatured whey protein (Fig.
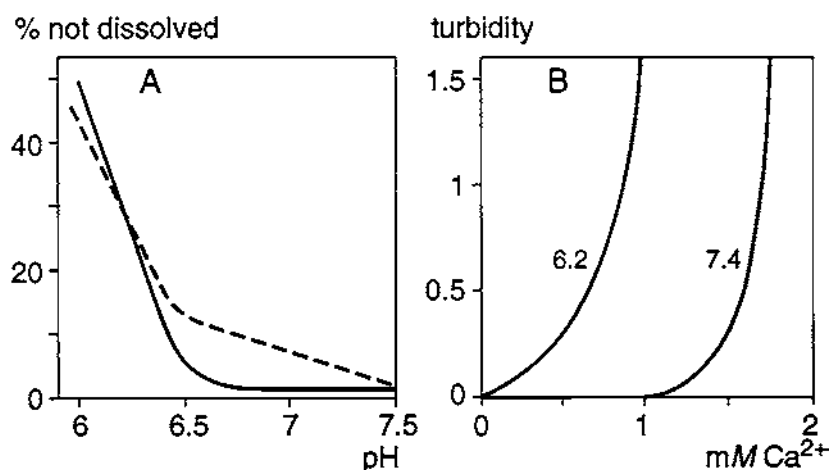
FIGURE 18.1 Solubility of β-lactoglobulin (———) and of purified whey protein (–––) as a function of the pH and the concentration of dissolved calcium during heating for 10 min at 80°C. (A) Part of the protein that can be separated centrifugically; low Ca concentration. (B) Turbidity of 1% solutions as a measure for the protein aggregation; parameter is the pH. Approximate results after J. N. de Wit, *Neth. Milk Dairy J.* **35** (1981) 47.

18.2). The product obtained is washed and dried, e.g., in a drum drier. This protein preparation is called lactalbumin, not to be confused with the serum protein α-lactalbumin. It contains little or no proteose peptone, caseinomacropeptide, or NPN. The high lactose content and the slowness of drying are responsible for extensive Maillard reactions. Lactalbumin is insoluble in water.

18.2.4 Coprecipitate

In a similar way as described for isolation of lactalbumin from whey, coprecipitate can be isolated from acidified skim milk or buttermilk (see Fig. 18.2). Most but not all of the milk proteins (except proteose peptone) are isolated in an insoluble state. The protein is easily digested and has a high nutritive value. Most preparations contain much calcium. Coprecipitate may contain far less Maillard products than lactalbumin because its sugar content is lower. This definitely holds if washing and drying are carefully carried out.

18.2.5 Separation and Modification

Individual proteins can be isolated by using laboratory techniques such as gel filtration and column electrophoresis. Salting-out or precipitation steps with etha-
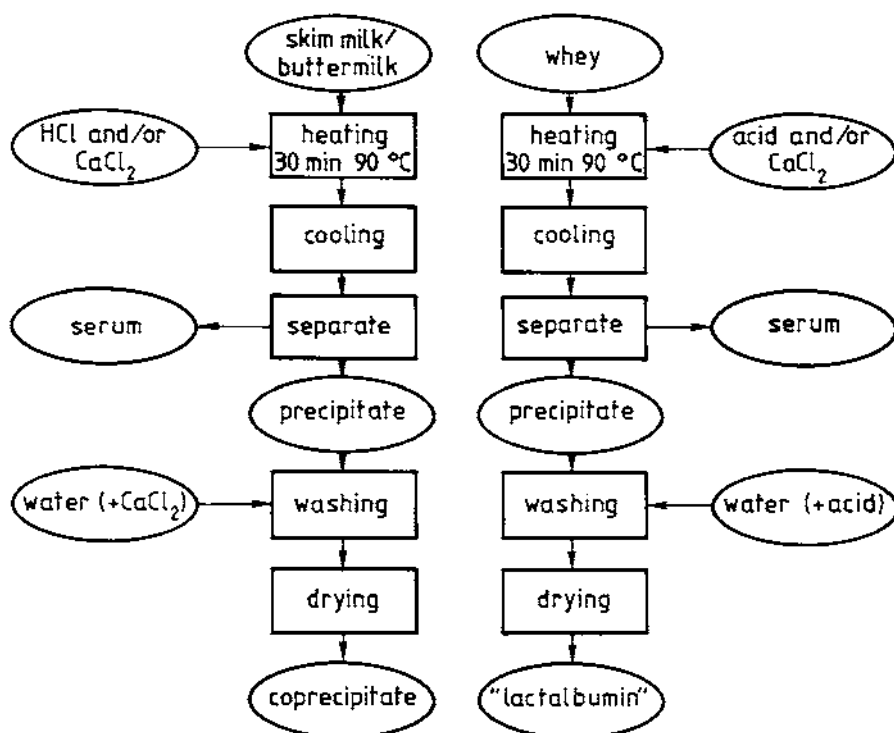
Figure 18.2 Examples of the manufacture of coprecipitate and lactalbumin.

nol or with specific salts (e.g., Ca salts) are sometimes used for protein separation. NIZO has developed a manufacturing process to purify whey. At a sufficiently low ionic strength and a suitable pH, the immunoglobulins precipitate specifically, thereby also removing most of the lipids (fat globules) and particulate material. After ultrafiltration of the supernatant a very pure preparation, largely consisting of β-lactoglobulin, α-lactalbumin, and serum albumin, is obtained.

Modification of the proteins is mostly restricted to their partial hydrolysis. Chemical hydrolysis, using acid or alkali, and enzymic hydrolysis are applied. Partially hydrolyzed casein can be used in foam products if the foaming liquid is highly viscous.

18.3 FUNCTIONAL PROPERTIES

A number of functional properties are usually distinguished to define the suitability of protein preparations for their use in foods. It often concerns some physical

parameter, determined in a solution of the preparation. The properties involved can, however, closely depend on conditions such as pH, ionic strength, temperature, water content, and presence of reagents. Various authors have made their own classification; ours is as follows.

18.3.1 Solubility

Good solubility is usually required for preparations used in liquid foods (e.g., beverages). Good solubility is a prerequisite for some other properties like emulsifying and foaming power. For use in dry foods such as biscuits a fairly complete insolubility is desirable, as applies for rennet casein, coprecipitate, and lactalbumin.

The concept of ''solubility of protein preparations'' is poorly defined. Often, a dispersion of the product is made in water or in a buffer solution. The mixture then is stirred in a standard way and the fraction of the protein that does not sediment in a centrifuge test is determined. A result of ''50% soluble'' can obviously imply that half of the protein dissolves well in a little bit of water, whereas the remainder is insoluble; in other words, dilution then would not cause an increased dissolution of protein. The result can, however, also mean that all of the protein dissolves if the double amount of water is used. The truth is mostly somewhere in between. Apart from that, the solubility closely depends on the experimental conditions. Examples for Na-caseinate are given in Figure 18.3, for whey protein concentrate in Figure 18.6. At low pH, caseinate can be salted out at quite low NaCl concentrations, where undenatured whey proteins stay in solution.
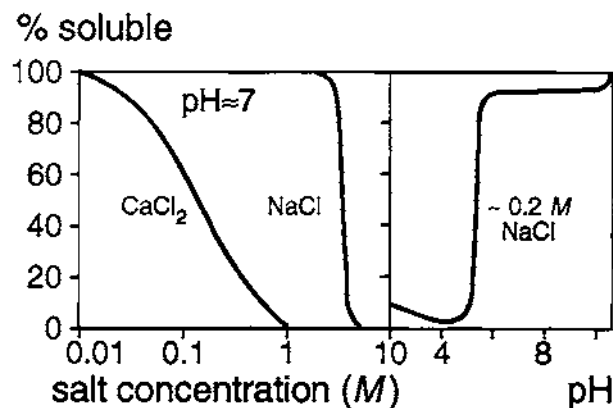
FIGURE 18.3 ''Solubility'' of Na-caseinate (3% in water) as a function of salt concentration and pH.

18.3.2 Gelling Properties

For some applications, a protein solution or dispersion should become a gel or a solid mass; syneresis usually is undesirable. Whey proteins in solution are ''heat setting'' because they become insoluble by heating if the pH is low, and also at a higher pH if $[Ca^{2+}]$ is not too low (Fig. 18.1). The whey protein concentration has to be high, i.e., several percent, for a gel to be formed. Gels made at a pH close to the isoelectric point have a fairly coarse microstructure, whereas at higher pH more fine-stranded gels are formed. Caseinate solutions can be made to gel by acidification, renneting, or with $CaCl_2$, but the gels often show syneresis. Ca-caseinate can be made insoluble by the addition of orthophosphate, and this is applied in spinning and extrusion of concentrated solutions.

18.3.3 Swelling

Swelling is often erroneously defined as water binding or hydration. However, it refers to the quantity of water or, more precisely, aqueous solution that a given quantity of protein can imbibe (retain mechanically). Swelling greatly depends on conditions. Casein tends to swell more at a higher pH, a lower Ca^{2+} activity, and a lower temperature. Serum proteins show little swelling; they are globular proteins. After denaturation, serum proteins imbibe a similar amount of water as micellar casein, say 2 g per gram of protein.

18.3.4 Viscosity of Solutions

This is primarily a function of protein concentration, state of swelling of the protein molecules, and any aggregation (see also Section 3.3). The viscosity of solutions of whey protein concentrates is low, that of CMC–whey protein complex is far higher. Figure 18.4 shows examples for Na-caseinate. Ca-caseinate gives less viscous solutions than Na-caseinate.

18.3.5 Emulsifier Properties

Often, some ''emulsifying power'' is defined by determining the amount of oil that can be emulsified in a certain protein solution by vigorous stirring, without immediate oiling off. However, that makes little sense unless the experimental conditions mimic the practical situation precisely. The following aspects should be distinguished:

    a. The protein layer formed around the oil globules has a certain thickness or *protein load* (mass of protein per unit oil surface area). The load determines the amount of protein needed to produce an emulsion and closely depends on the solubility of the protein. Globular proteins (occurring, for instance, in whey) and well-dissolved Na-caseinate give
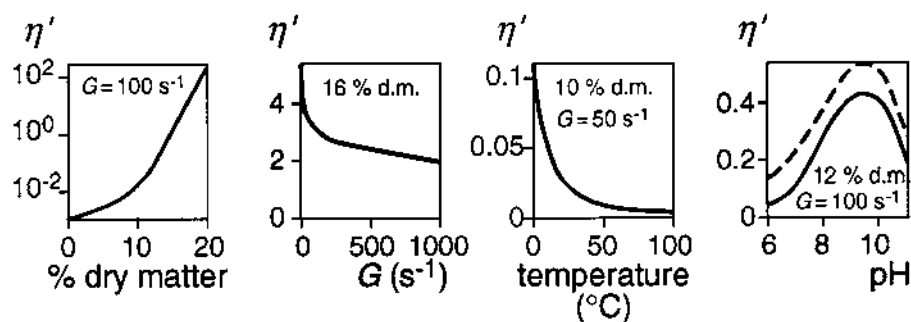
FIGURE 18.4 Apparent viscosity (η', Pa · s) of solutions of Na-caseinate in water (——) or 0.2 M NaCl (– – –), at pH $\approx$ 7 and room temperature, unless stated otherwise. *G*, shear rate.

a protein load of some 2.5 mg · m^{-2}, while Ca-caseinate and micellar casein give far higher values, and poorly soluble proteins cannot be used for emulsification. Obviously, the load depends also on the amount of protein available per unit oil surface area created (see Fig. 18.5) and on factors affecting the conformation of the protein (pH, ionic strength, specific salts, temperature, heat treatment).

b. The *size of the globules* obtained depends on emulsifying conditions and on the concentration and type of the protein. Figure 18.5 gives examples. To obtain the same specific surface area, more protein is needed if its molar mass is higher or if it is in an aggregated state, but if the protein is relatively abundant, the differences between various proteins are small.

c. The stability of the oil droplets toward *coalescence* or partial coalescence closely depends on the globule size and on the protein load. If a small amount of protein is available, the globules formed are large and the protein load is small. As a result, the globules are relatively unstable. Na-caseinate gives very stable emulsions if the protein load exceeds 2 mg · m^{-2}, but it cannot be used for emulsification at its isoelectric pH. A Na-caseinate emulsion can, however, be made at pH 7 followed by acidification, without coalescence of the droplets occurring (see also aspect d). Na-caseinate can also be used to yield emulsions that remain stable during spray drying, storage of the powder, and redissolution.

Emulsions made with soluble whey proteins are stable except when pH is 3.5–5.5 (Fig. 18.6). Heat-denatured whey proteins often are poor stabilizers of emulsions because of their low solubility, though denaturation at high pH is not so detrimental.
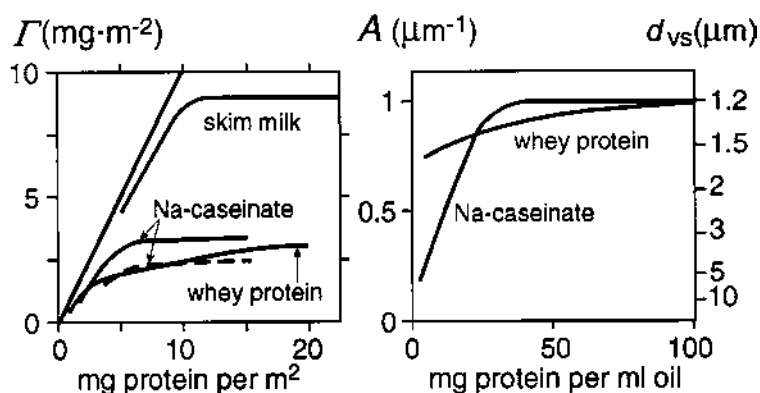
FIGURE 18.5 Emulsifying properties of milk proteins. (left) Surface load (Γ) as a function of protein initially available in the solution per m² oil surface area after emulsification. Na-caseinate in 0.2 M NaCl (————) and in water (–––). The straight line gives the Γ that would be acquired if all protein available were adsorbed. (right) The specific surface area (*A*) obtained as a function of available protein per ml of oil; volume fraction of oil = 0.2. Approximate examples at otherwise constant conditions.

    d.   The emulsion globules *aggregate* when changed conditions cause the protein in the surface layer to become insoluble. Globules coated with caseinate flocculate on adding rennet, bringing to a pH of about 4.5, adding much $CaCl_2$, or prolonged heating at high temperature. (Emulsions stabilized with whey protein coalesce at high temperature, probably due to heat denaturation of the protein.) Under certain conditions homogenization clusters can be formed (Section 8.7).

18.3.6 Foaming

The solubility of a protein should be good and its lipid content virtually nil for a solution of the protein to foam. A high viscosity of the solution is desirable to slow down drainage of the foam. Na- and K-caseinate give copious and fairly stable foams if no lipid is present. Ca-caseinate is less suitable. Partly hydrolyzed casein gives stable foams if the solutions have a very high viscosity. Whey protein concentrates, if in an undenatured state, also give copious and stable foams in the absence of lipids (Fig. 18.6). Whey proteins heat-denatured at high pH (e.g., 20 min at 85°C, pH 7.5), without much calcium being present, give somewhat less foam (overrun is, say, 1000% for a 10% solution), but foam stability is better. Furthermore, a suitable concentrated whey protein solution can be foamed and
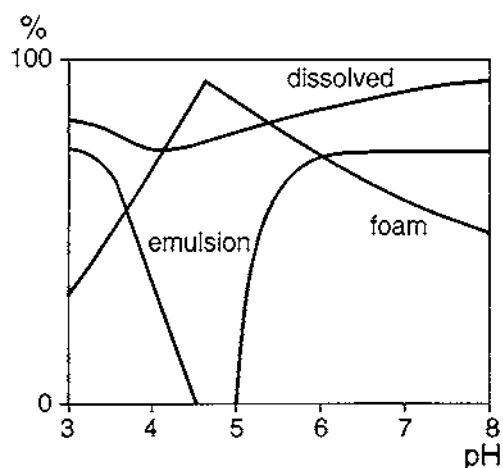
FIGURE **18.6**   Some functional properties of whey-protein solutions as a function of the pH. Percentage of dissolved protein after high-speed centrifugation of a 1% solution. Amount of foam formed after whipping of a 10% solution (arbitrary scale). Percentage of the oil that does not cream in a centrifuge test on an emulsion in a 4% protein solution. Approximate examples after J. N. de Wit, *Zuivelzicht* **67** (1975) 228; and after J. N. de Witt et al., *Zuivelzicht* **68** (1976) 442.

subsequently heated so that the whey protein gels and a solid foam is formed, e.g., a meringue.

18.4   OTHER PROPERTIES

The nutritive value of a protein depends on its digestibility, which is excellent for most milk protein preparations, and on its amino acid pattern, which is good in casein, better in whey proteins, and still better in blends of both. Whey protein concentrates are widely used in baby foods to give them a composition (also as to their protein pattern) more like that of human milk, which contains somewhat over 0.3% casein and somewhat over 0.5% whey protein. For babies, such foods are preferred over cows' milk because of the better amino acid pattern, the poor rennetability (soft curd), and the lower kidney load.

Loss of available lysine may widely vary, and increases during storage according to such conditions as lactose content, water activity, and temperature. In a well-made and well-stored product the loss may be negligible.

Casein preparations can undergo severe heating during manufacture, so

they may contain appreciable quantities of lysinoalanine (Section 6.2), i.e., 100–500 mg/kg of protein, especially if pH is high during heat treatment. This substance is not toxic for humans (though it is for rats), but its concentration should be kept low.

Pure proteins have no flavor. Other constituents and protein deterioration are responsible for any off-flavor the preparations may have. Deterioration is like that of other dry milk products (Section 17.7). Dissolved whey protein concentrates are prone to development of ''sunlight flavor.''

SUGGESTED LITERATURE

• Several aspects of protein preparations are discussed in:
> P. F. Fox, ed., *Developments in Dairy Chemistry*, Vol. 4, *Functional Milk Proteins*, Applied Science, London, 1989.

19

Butter

19.1 DESCRIPTION AND MANUFACTURE

19.1.1 Description

Butter is generally made from cream by means of churning and working. It contains somewhat over 80% fat, which is partly crystallized. The churning proceeds most easily with sour cream, at a temperature of around 15–20°C. Therefore, butter typically is a product originating from regions of temperate climate. In addition to accumulated practical experience, a good deal of science has now been incorporated in butter making, enhancing the shelf life and the quality of the product and the economy of manufacture.

Some variants occur: butter from cultured (soured) or from sweet cream; butter with or without added salt. Formerly, the salt was applied as a preservative; nowadays it is mainly added for the flavor. Previously, the souring of the cream inevitably occurred (due to the duration of the gravity creaming), and now it is practiced intentionally. It enhances the keeping quality (although this hardly makes a difference when applying modern technology) and it greatly affects the flavor.

Following are the most important specific requirements for the product and its manufacture.

a. *Flavor*. Off-flavors of the fat are to be avoided, especially those caused by lipolysis, but also those due to volatile contaminants. The latter mostly dissolve readily in fat; notorious examples are off-flavors caused by feeds like silage and *Allium* species. If the cream is heated too intensely the butter gets a cooked flavor. Moreover, much attention has to be paid to the souring. This processing step is discussed in Chapter 11, but here we want to note that the formation of the so-called

aroma by heterofermentative lactic acid bacteria is essential. The most important aroma substance is diacetyl; not only is its formation important but also its persistence, since some starter bacteria can reduce the diacetyl again. The homofermentative *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* also forms diacetyl, but some strains produce so much acetaldehyde that the butter develops a yogurt flavor.

b. *Shelf life*. Spoilage by microorganisms may cause several off-flavors (putrid, volatile acid, yeasty, cheesy, rancid); in cultured cream butter it usually involves molds and yeasts, the pH of the moisture being too low (~4.6) for bacterial growth. Lipolysis causes a soapy-rancid flavor; no lipases formed by psychrotrophs should be present in the milk. Furthermore, autoxidation of the fat can also occur, especially at prolonged storage, even at a low temperature ($-20°C$), leading to a fatty or even a fishy flavor.

c. *Consistency*. Butter derives its firmness largely from fat crystals that are aggregated into a network. Butter should be sufficiently firm to retain its shape; likewise, oiling off (i.e., separation of liquid fat) should not occur. On the other hand, the butter should be sufficiently soft as to be easily spreadable on bread. This causes great problems because the firmness and the spreadability closely depend on the composition of the fat and on the temperature.

d. *Color and homogeneity* are mostly easy to get right.

e. *Yield*. Losses of fat occur at skimming (in the skim milk) and at churning (in the buttermilk). If the water content is below the legal limit (e.g., 16%), this also means a loss of yield.

f. The byproduct *buttermilk* is sometimes desirable, but it is often undesirable because of insufficient demand. Sour cream buttermilk is only applicable as a beverage (or is used for cattle feeding purposes), but it will keep poorly due to rapid development of an oxidized flavor. Sweet cream buttermilk can more readily be incorporated in certain products.

19.1.2 Manufacturing Scheme

Figure 19.1 gives a schematic example of more or less traditional butter making from sour cream. Figure 19.2 shows a schematic representation of the physical changes involved.

The *skimming* is mainly done for economical reasons: reduction of fat loss (e.g., the fat content of buttermilk is 0.4%, that of skim milk is 0.05%; this means that removal of 1 kg of skim milk from the liquid to be churned will result in an additional yield of about 4 g of butter); reduction of the size of the machinery (especially the churn); reduction of the volume of buttermilk. Hence, a high fat content of the cream (e.g., 40%) has advantages, also because it counteracts de-
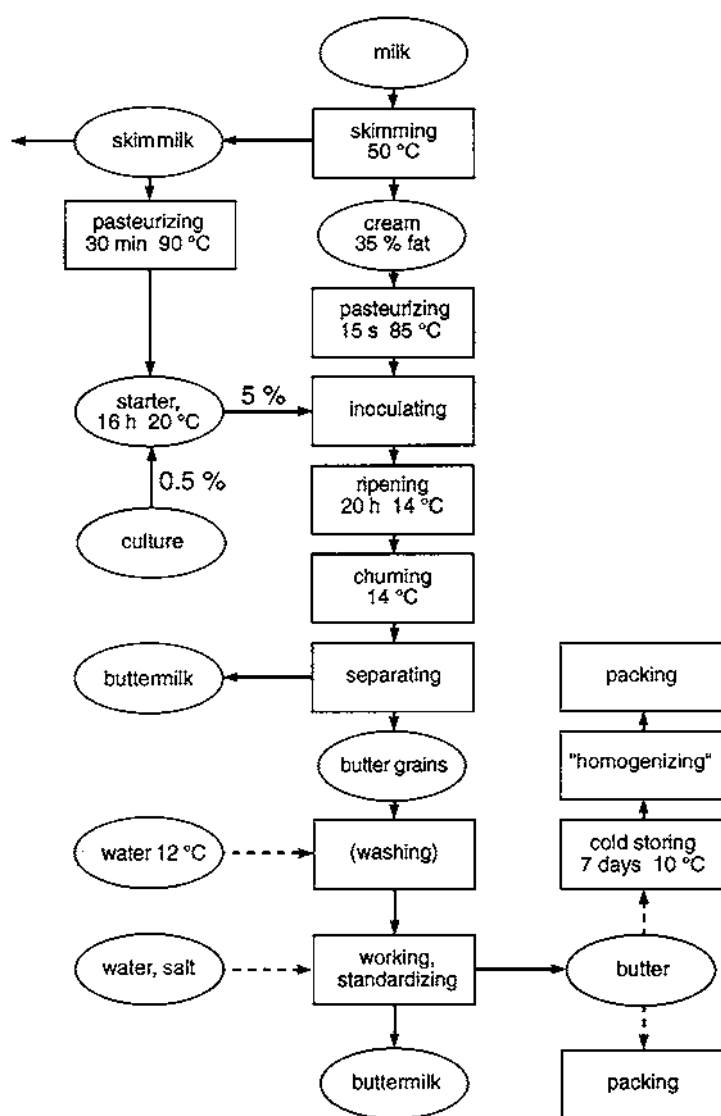
FIGURE 19.1 Example of butter making from ripened cream.

FIGURE 19.2   Stages in the formation of butter. Greatly simplified, not to scale. Black is the aqueous phase; white is fat. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

velopment of off-flavors (see Section 19.2.3). If a continuous butter-making machine is used, the fat content of the cream is often taken even higher, i.e., up to 50%.

In some countries the farmers skim the milk and deliver the cream to the dairy. Such cream is often in a poor condition, i.e., more or less turned sour and having off-flavors. Generally, such a cream has to be neutralized, e.g., with NaOH or $NaHCO_3$, to allow its pasteurization. The cream may also be washed, i.e., diluted with water and reseparated, to remove undesirable flavors; however, this is not very effective.

Pasteurization serves to kill microorganisms, to inactivate enzymes, to make the cream a better substrate for the starter bacteria, and to render the butter more resistant to oxidative deterioration (see Section 19.2.3). Overly intense heating causes a cooked or gassy flavor. Sometimes the cream is pasteurized in a vacreator, which implies that the hot cream is put under vacuum to cool, due to which some off-flavors are (partly) removed.

The *starter* should be ''aromatic,'' i.e., produce aroma substances and retain these. To that end it can be necessary to aerate the starter for a while at the end of the incubation period, e.g., by stirring. The starter bacteria should be able to grow fairly fast at low temperature.

The purpose of *ripening* is to sour the cream and to crystallize the fat. Without solid fat, churning is impossible, and too little solid fat goes along with excessive fat loss in the buttermilk. The way of cooling (temperature sequence) affects the butter consistency (see Section 19.2).

The *churning* is in most cases achieved by beating in of air (see Section 19.1.3). It can be done in a churn, mostly consisting of a large vessel (tub, cylinder, cube, double-ended cone) with so-called dashboards, which is partly (at most half) filled with cream, and which is rotated at several revolutions per minute (rpm). The churning then takes, say, 20 min. There are also churns with a rotary agitator (e.g., 20 rpm). The latter principle is also applied in the frequently used continuous butter-making machine according to Fritz (see Fig. 19.3). Here the paddle turns very quickly (500–3000 rpm) and the cream stays in it for less than
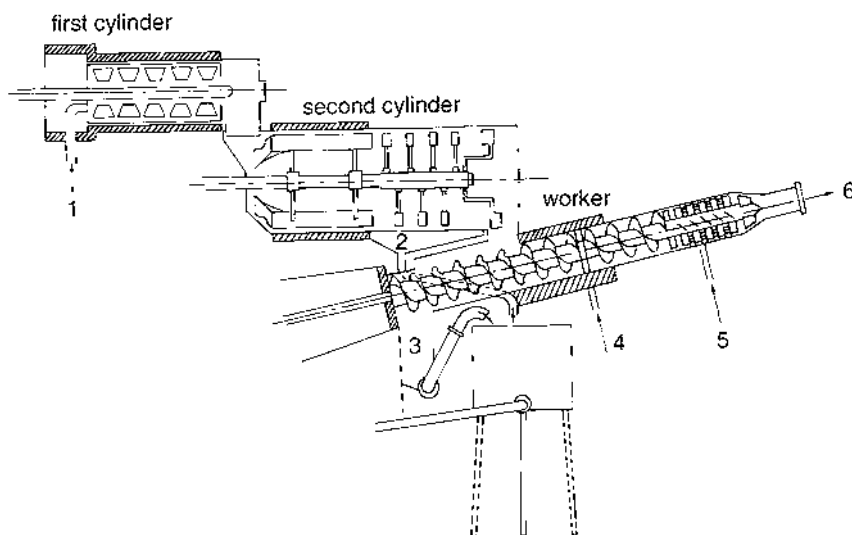
FIGURE 19.3 Example of a continuous butter-making machine according to Fritz; highly simplified diagram. The cream enters at (1) and is very intensively churned in the first cylinder (turning speed of beater, e.g., 2000 rpm), yielding very fine butter grains. In the second cylinder (say, 30 rpm) the grains are churned into larger ones, allowing the buttermilk to drain off via a sieve (2). The grains fall in the worker, where they first are kneaded together by the worm, with the residual buttermilk being drained off (3). The mass may be chilled with water (4). The butter is now squeezed through a series of perforated plates and leaves the machine as a strand (6). To adjust the butter composition additional water, brine, etc., can be incorporated during working (5). Some machines are equipped with two worker sections in series.

1 min. For that purpose high-fat cream has to be used, i.e., about 50% fat. These machines can have a very great capacity.

The churning should proceed rapidly and completely (low fat content in the buttermilk) and the formed butter grains should have the correct firmness to allow for efficient working. The size of the butter grains can be varied by continuing the churning for various lengths of time after grains have formed. Very fine butter grains (say, 1 mm) are hard to separate from the buttermilk, especially in continuous machines.

If the butter grains are not too large their firmness can to some extent be affected by *washing*, i.e., via the wash water temperature. The washing consists of mixing the butter grains with water, after which this again is drained off. It reduces the dry matter content of the butter moisture. Formerly, washing was

applied to improve the keeping quality of the butter, but nowadays it is only done to control the temperature, if needed.

The *working* (kneading) is meant to render the butter grains into a continuous mass, to finely disperse the moisture in the butter, to regulate the water content, and, if desired, to incorporate salt (see Section 19.1.4). Working consists of deforming the butter. This can, for instance, be achieved by squeezing the butter through rollers, by allowing it to fall from a height (in the modern churn-and-workers), or by squeezing the butter through perforated plates (in the continuous machines). During the working the water content is regularly checked and, if need be, additional water is added to arrive at the accepted standard value.

The butter can now be immediately *packed*, e.g., in retail package. Often one wants the butter after the working to be soft enough to be pumped from the churn-and-worker by means of a suitable positive pump. Sometimes, the butter is allowed to set (see Section 19.2.2) or it is for another reason kept for some time before packing. It is then too firm to pass through the packing machine, and it must be passed through a butter homogenizer to soften it; this may also prevent the moisture dispersion from becoming too coarse during packing.

19.1.3   The Churning Process

The instability of fat globules and their interactions with air bubbles are discussed in Section 3.1 (see especially Fig. 3.7).

During churning, air is beaten into the cream and is dispersed into small bubbles. The fat globules touch these bubbles, often spread part of their membrane substances and some of their liquid fat over the air–water interface, and become attached to the bubbles; one bubble ''catches'' several globules. This resembles flotation, although in true flotation the foam is collected. In the churning process, however, the air bubbles keep moving through the liquid and collide with each other. They thus coalesce, and in this way their surface area diminishes. As a consequence, the adhering fat globules are driven toward one another. Now the liquid fat acts as a sticking agent and the fat globules are clumped together. In this way small fat clumps are formed. All these changes are illustrated in Figure 19.4.

The clumps, in turn, participate in the churning process, resulting in still larger clumps. When the clumps become larger, direct collision between them increasingly occurs, and the clumps now grow without the air bubbles any longer playing an important part. Flotation thus predominates initially and plain mechanical clumping later on. In addition, more and more liquid fat and surface layer material is released (it first spreads over the air bubbles and partly desorbs as soon as the bubbles coalesce); this is called *colloidal fat*, and it consists of tiny liquid fat globules and membrane remnants. Toward the end of the churning little foam has been left, presumably because too few fat globules remain to cover the
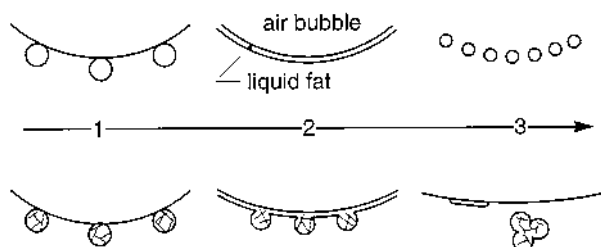
FIGURE 19.4 Schematic representation of the interactions between fat glob-
ules and air bubbles during churning. The fat is liquid and it is disrupted by
beating in of air (top). The fat globules contain solid fat and form fat clumps
(bottom).

air bubbles and thereby stabilize these bubbles. These changes are illustrated in
Figure 19.5.

Several factors affect the churning process, i.e., both its rate and its effi-
ciency. Figure 19.6 gives examples. Type and filling level of the churn naturally
have effect, as well as the turning speed of the churn. The churning time decreases
with increasing fat content, but less sharply than would be expected on account
of the increasing probability of collision between fat globules (the churning time
then would vary inversely with the square of the fat content). Obviously, the
flotation churning is a very efficient process; even milk can be churned readily,
and only if very high-fat cream is used the fat content of the buttermilk increases.
The influence of the size of the fat globules is as expected. Homogenized milk
cannot be churned.

The proportion of solid fat is crucial (see Fig. 3.6A). If the fat is fully
liquid, a kind of homogenizing rather than churning occurs (Fig. 19.4, upper
row). Also, if the globules contain very little solid fat, then the cream does not
churn; the ''clumps'' formed are soon pulled to pieces. But for the rest, the higher
the proportion of solid fat, the slower the churning, and the lower the fat content
in the buttermilk. If the fat globules contain relatively little liquid fat, they can
still be attached to the air bubbles and the first stages of the churning, where
flotation predominates, do occur; but mechanical clumping hardly takes place
and one should raise the temperature to allow the formation of butter grains.

The temperature will therefore have a considerable effect on the churning,
but so does the temperature history (see Section 2.3.4). If the precooling is not
sufficiently deep, supercooled fat globules will still be present, and particularly
the fat content of the buttermilk will increase. Some properties of the milk plasma,
particularly its acidity, also affect the churning process, but in most cases the
explanation is not clear.
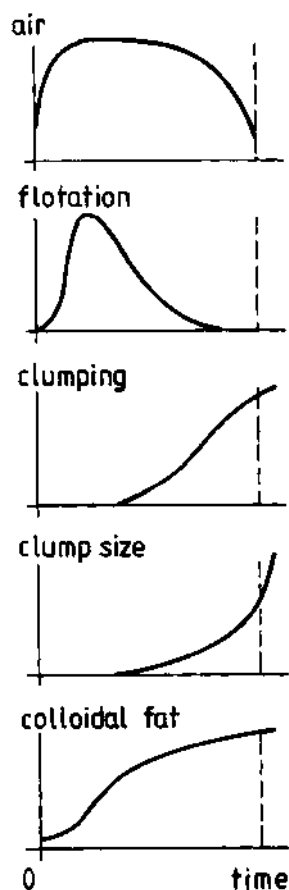
FIGURE 19.5 Events taking place during traditional churning. Amount of air entrapped in the cream; rate of flotation (extent to which fat globules attach to air bubbles); rate of clumping; size of the clumps or butter grains; amount of fat in a colloidal state (i.e., not recoverable by centrifugation). The broken line indicates the point of ''breaking'' (formation of clearly visible butter grains). Approximate examples. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

Note in Figure 19.6 that rapid churning mostly coincides with a high fat content in the buttermilk (except with regard to the influence of the fat globule size). If much liquid fat can spread over the air bubbles, disruption of fat globules occurs. Furthermore, the smallest fat globules in particular may easily escape the churning process if it proceeds very rapidly. Sweet cream buttermilk can be
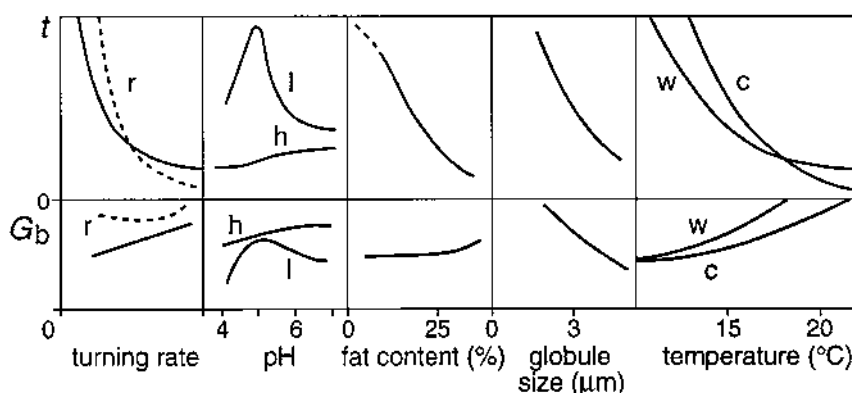
FIGURE 19.6 The effect of some variables on the churning time (*t*) and the efficiency (as % fat in buttermilk, G_b) in a traditional churn. Factors are turning rate of churn or agitator; pH of the cream; fat content of the cream; average fat globule size; churning temperature. r, churn with rotary agitator; l, low (~11°C); h, high churning temperature (~19°C); c, cream kept cool before the churning; w, cream kept warm before bringing it to churning temperature. Approximate results. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

separated centrifugically (sour buttermilk cannot), but its fat content remains rather high (e.g., 0.2%) due to the ''colloidal'' fat.

The churning proceeds very fast in a continuous butter-making machine; the beater turns at a very high rate. Accordingly, the fat content of the buttermilk tends to be high, unless the cream is deeply precooled (4°C) and is churned at low temperature (8–12°C). A high-fat cream then is required for sufficiently rapid churning. Presumably, mechanical clumping is more important here than in a classic churn. A high-fat cream can even be churned by means of a rapidly rotating paddle without the beating in of air, with the churning time being mostly somewhat longer, however, and the fat content in the buttermilk slightly higher.

19.1.4 Working

A partial phase inversion occurs during the churning; in the butter granules a continuous fat phase has developed (see Fig. 19.2). But in the whole mass of butter grains the aqueous phase is still continuous. The working accomplishes a further phase inversion. In this stage excessive moisture is squeezed out and remaining moisture droplets are disrupted into smaller ones. This does not concern the very small moisture droplets that are left between individual clumped fat globules; these are too small (on average about 2 μm) to be disrupted by the working.

During the working the butter is deformed, and hence velocity gradients (G) occur. The deformation (flow) causes a shear stress $G \times \eta$, where η is the viscosity. The flowing mass, roughly a mixture of liquid fat and aggregated crystals, does not have a true viscosity, but due to the presence of the crystals the effective viscosity (η_{eff}) is high. The flow also exerts a stress on the moisture droplets, thereby deforming them. If the stress involved exceeds the Laplace pressure of a droplet ($4\gamma/d$, where d = droplet diameter and γ = interfacial tension oil-plasma, amounting to about 15 mN · m^{-1}), the droplet is disrupted. This is illustrated in Figure 19.7A and B. Disruption more effectively occurs during extensional flow than during simple shear. Extensional flow always occurs during working. Obviously, at a higher velocity gradient (more intensive working) or at a higher effective viscosity of the butter (greater amount of solid fat, thus for instance lower temperature), smaller droplets can result. (*Note*: In some, but not all, practical situations essentially a certain stress is applied. A higher viscosity then does not result in a higher shear stress because a proportional decrease in velocity gradient occurs.)

During the working of butter, the velocity gradient varies widely from place to place and from one moment to the other. Figure 19.7D (left) illustrates the velocity pattern in case of true Poiseuille flow. It shows that the velocity gradient, proportional to ctn α, depends closely on the place. The differences may even be much greater due to the fact that the mass of oil and crystals shows a kind of plug flow (Fig. 19.7D, right). This is because the mass has a yield stress; when the butter flows along a wall (e.g., through an opening), the shear stress in the mass may be largest near that wall so that the mass yields there, thereby locally decreasing the effective viscosity. In other words, a small proportion of the butter deforms with a strong velocity gradient; a larger proportion with a very weak gradient.

Because of a velocity gradient, the droplets collide with one another and can coalesce, assuming that the shear stress is small enough to prevent redisruption. This is shown in Figure 19.7C. The collision frequency of a droplet (diameter d_1) with other droplets (number per unit of volume N_2, diameter d_2) is about equal to $(d_1 + d_2)^3 N_2 G/6$. The probability for coalescence will thus be greater for larger droplets, but such droplets will be easier disrupted as well. A kind of steady state of disruption and coalescence now develops (assuming that G and η_{eff} remain constant), and this is reflected in a certain droplet size. But a wide droplet size distribution will result because, as mentioned, G varies widely, so that during working disruption predominates in some places, and coalescence in others. An example is given in Figure 19.8.

The above-mentioned aspects can help to find out the actual processing conditions needed to obtain a product of desired properties. One additional aspect should be mentioned. The above (partial) plug flow especially occurs at low temperature (much solid fat), so that a very wide size distribution with numerous
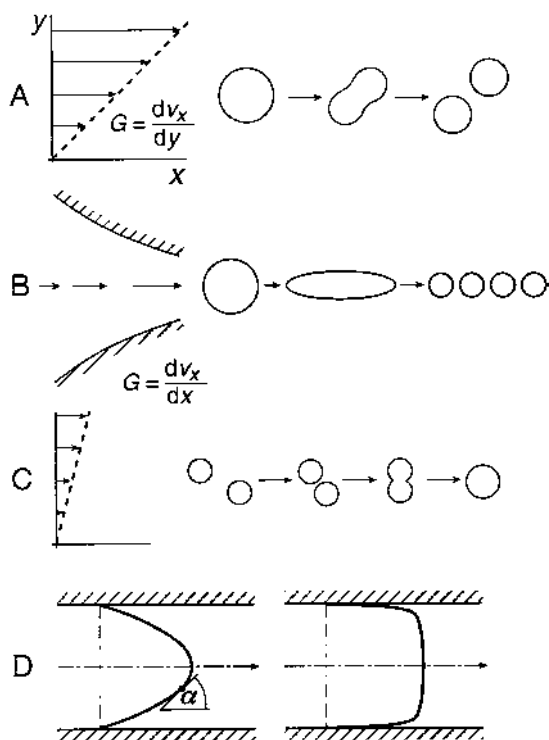
FIGURE 19.7 Diagrammatic representation of the disruption and coalescence of moisture droplets during the working of butter (or margarine). (A) Disruption in a flow with simple shear, i.e., velocity gradient normal to the direction of the flow. (B) Disruption in a converging flow (= extensional flow), i.e., velocity gradient in the direction of the flow. (C) Encounter and coalescence of (small) droplets in flow; shear stress too small to disrupt the droplets. (D) Velocity patterns in Poiseuille flow (left) and in partial plug flow (right).

droplets is obtained, despite the high effective viscosity. Increasing the working speed causes the droplets to become smaller; the butter becomes "dry." Likewise, in this way moisture can be incorporated into the butter. When working at a very slow speed, larger droplets emerge again, especially at low temperature; the butter becomes "wet," i.e., visible droplets appear. In that way, excessive moisture can be worked out of the butter. The butter can also readily become wet during repacking in retail packages, where weak velocity gradients prevail.

The moisture dispersion, i.e., the fineness of the droplets, is of great impor-
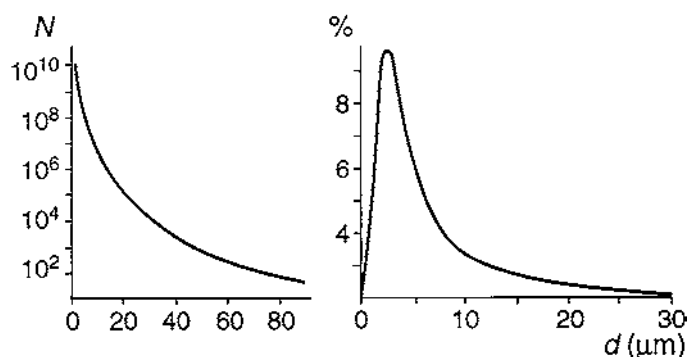
FIGURE 19.8   Example of the size frequency distribution of moisture droplets in a well-worked butter. *N* is number frequency (per ml per μm class width); % refers to the volume of the moisture per μm class width; *d* is droplet diameter. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

tance for the keeping quality. Contamination by microorganisms cannot be fully prevented; because of this, butter can spoil readily. But if there are, say, 10^3 microorganisms per ml and 10^{10} moisture droplets (see Table 19.1), then only a negligible part of the moisture would be contaminated; since the microorganisms cannot pass from droplet to droplet, the spoilage will be negligible. Naturally, it is especially the large droplets that become contaminated. The fraction of the moisture being contaminated is proportional to the colony count and approximately to the volume-average volume $(\pi/6) \sum N_i d_i^6 / \sum N_i d_i^3$ of the droplets. If some large droplets (''free moisture'') are left in the butter, growth of microorganisms readily occurs. This is illustrated in Figure 19.9.

The smaller the droplets, the paler the butter due to the stronger light scattering. Apart from that, the color is mainly determined by the β-carotene content. Sometimes coloring matter is added.

The smaller the droplets, the flatter the taste of the butter: salt as well as ''aroma'' are much better perceived if the butter is slightly ''wet''. Hence, working dry should not be exaggerated. Especially in the continuous machines the working (squeezing through a set of perforated plates) is very intense. By this type of working many fat globules also are fragmented (see Section 19.2), and the butter becomes somewhat ''greasy'' in consistency. All this does not alter the fact that in many cases the problem is to obtain sufficiently fine droplets rather than the reverse.

Figure 19.10 serves to illustrate the approximate influence of average droplet size on the mentioned quality aspects. It follows that there is an optimum droplet size.
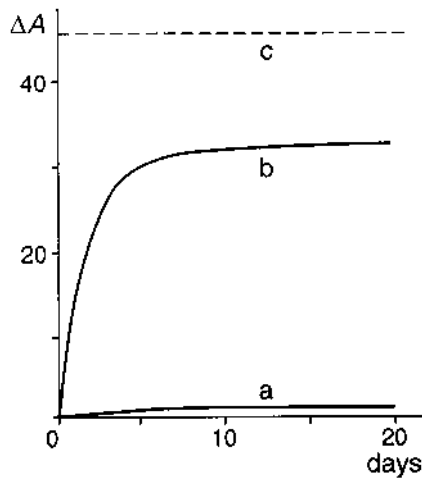
FIGURE 19.9 Increase in acidity (ΔA, mM) of the serum of butter kept at 17°C. Just before churning, 10% starter had been added to the sweet cream. (a) Butter worked until "dry." (b) Butter worked less well. (c) Final ΔA expected if all droplets were to acidify. Data provided by Mulder and Zegger, unpublished.
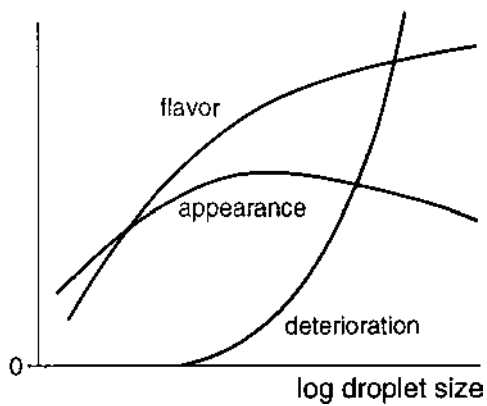
FIGURE 19.10 Intensity of perceived flavor (cultured cream butter), appearance score (color and gloss), and rate of deterioration by microorganisms as a function of the average aqueous droplet size of butter. Only meant to illustrate trends.

19.2   STRUCTURE AND PROPERTIES

19.2.1   Microstructure

Figure 19.11 and Table 19.1 give an impression of the microstructure of butter.

Striking (and an important difference with, for example, margarine) is the presence of many, partly intact fat globules. Their number depends on the way of manufacture and it decreases, for instance, during intense working. Note that most crystals in the fat globules are tangentially arranged.

The continuous phase is liquid fat. Sometimes a continuous aqueous phase persists, especially in insufficiently worked butter. This aqueous phase partly passes through the surface layers of the fat globules. The fact that displacement of water through butter can occur generally has another cause: approximately 0.2% (v/v) water can dissolve in liquid fat, which implies that water can diffuse through the continuous oil phase.

The moisture droplets are not always equal in composition. Differences are found due to water addition, washing, and the working in of starter, salt, or brine.

fat globules   membrane   fat crystals   aqueous droplet   air cell
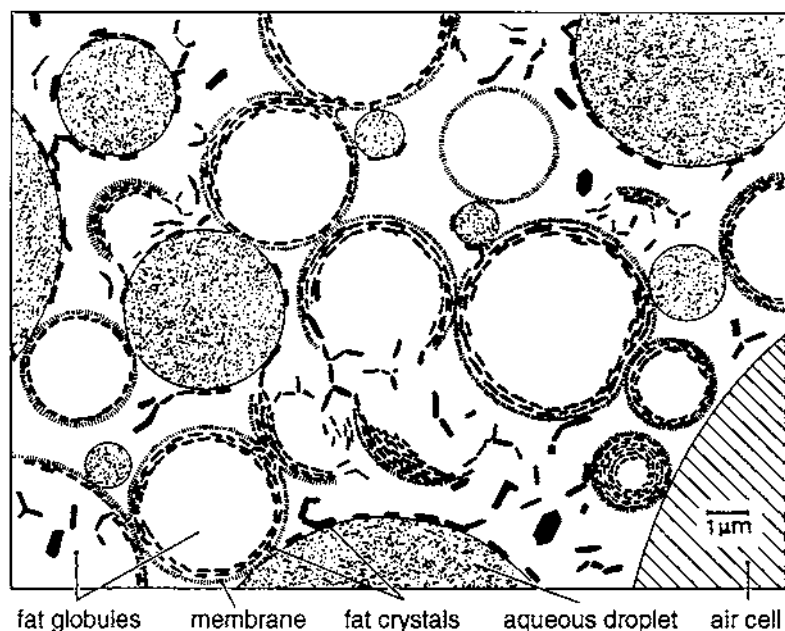
FIGURE 19.11   Butter microstructure at room temperature. Liquid fat is white. Membrane thickness is much (about 10 times) exaggerated. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

TABLE **19.1** Structural Elements of (Conventional) Butter

Structural element	Approximate number concentration (ml^{-1})	Proportion of butter (%, v/v)	Dimension (μm)
Fat globule[a]	10^{10}	10–50[c]	2–8
Fat crystal[b]	10^{13}	10–40[d]	0.01–2
Moisture droplet	10^{10}	15	1–25[c]
Air cell	10^{6}	~2	>20

[a] With (for the greater part) a complete membrane.
[b] At higher temperatures mainly inside the fat globules, at low temperatures forming solid networks.
[c] Closely depends on the working.
[d] Closely depends on the temperature.

Differences in osmotic pressure then cause a slow water transport to the most concentrated droplets. Hence, moisture droplets in the vicinity of a salt crystal mostly disappear, and the salt crystal leads to a large droplet; accordingly, the butter turns ''wet.''

The number and size of the fat crystals greatly depend on the temperature and on the temperature history. A considerable part of the crystalline fat may be inside the fat globules because during churning liquid fat is extruded from the globules, mainly by spreading over the air bubbles. But there are also crystals outside the globules and these aggregate to a continuous network and may grow together to form a solid structure, which is mainly responsible for the butter firmness. The crystals inside the globules do not participate in this network and, therefore, they hardly make the butter firmer. Because of this, butter generally contains more solid fat than margarine, when both products are equally firm; this results, in turn, in the butter feeling cooler in the mouth (due to the greater heat of fusion).

The crystals outside the fat globules thus make up a continuous network, in which part of the water droplets (often with crystals attached to their surface) and damaged fat globules may participate. This network retains the liquid fat as a sponge. Note that butter thus has at least two continuous phases (oil and fat crystals) and possibly a third one (aqueous). If the temperature increases many crystals melt and the network becomes less dense and coarser. Because of this, destabilization can eventually occur, i.e., the butter separates oil. Oiling-off occurs more readily (at equal solid fat content) if the crystals are coarser.

Air cells always occur in butter, unless the working is done in vacuum (which is possible in some continuous machines). Moreover, butter contains up to about 4% (v/v) of dissolved air.

19.2.2 Consistency

The rheological behavior of butter has many aspects. Butter should be sufficiently firm against sagging. It should be readily spreadable and thereby not too ''long'' (extensible) or too ''short'' (crumbly, flaky). It should be easily deformable in the mouth without being greasy, should melt rather quickly, and should thereby feel cool. A full rheological description of butter would be very complicated and cannot yet be given. Generally, we use the term firmness (also, but less correct, hardness) to denominate the main property. Firmness is a poorly defined quantity and depends on the type of deformation applied, on the time scale of the experiment, etc. In most cases, spreadability correlates rather well (negatively) with it.

The crystallization behavior of milk fat is briefly discussed in Section 2.3.

A fat (i.e., crystals in oil) usually is a plastic material; accordingly, one refers to its consistency, not its viscosity. At very low stress elastic properties predominate (the shear modulus equals, say, 1 MPa), but at higher stress σ the fat starts to flow; hence, it shows a yield stress. For still higher σ, the deformation rate is given by $\dot{\epsilon} \approx (\sigma - \sigma_B)/\eta_B$, where σ_B is the extrapolated or Bingham yield stress, and η_B the Bingham viscosity (thus not a true viscosity) (see Fig. 19.12A). σ_B is a reasonable measure of the firmness of a fat, though the value depends on the measuring conditions.

The main factors affecting the firmness are:

a. As mentioned in Section 2.3.4, the crystals aggregate into a network with meshes of the order of 1 to several μm in size. This gives the fat a certain elasticity at very small deformations. At greater deformation,
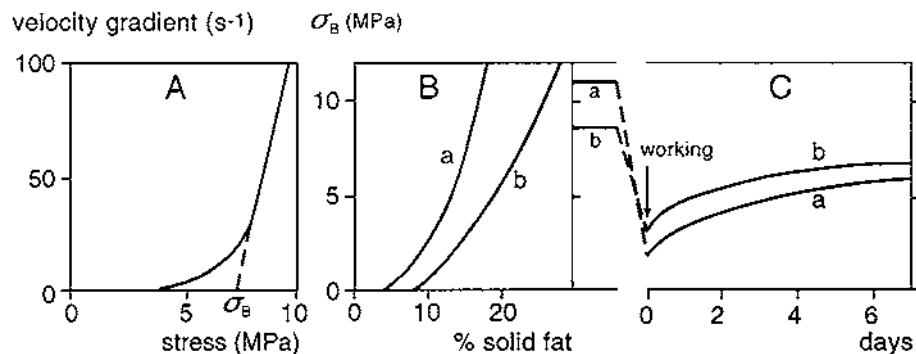
FIGURE 19.12   (A) Deformation rate of butter as a function of the stress applied. (B) Firmness as Bingham yield stress (σ_B) as a function of the percentage of solid fat. (C) Effect of working and of the length of time after working on σ_B. a = plastic fat or margarine; b = butter. Approximate examples. Mainly after P. Walstra and R. Jenness, *Dairy Chemistry and Physics* (New York: Wiley, 1984).

bonds have to be broken; this involves the most important contribution to the firmness of a freshly crystallized fat.
b.   As soon as aggregated crystals grow further (which may have several causes; Section 2.3.4) they sinter, i.e., grow locally together to form a solid structure (see Fig. 2.15), thereby very much increasing the firmness. At (large) deformation the solid structure has to be broken.
c.   The amount of solid fat: see Figure 19.12B. Hence, a very strong influence of the temperature.
d.   Size and shape of the crystals. Milk fat with 20% crystals in the form of large spherulites is still liquid, but with 10% of the fat in small crystals it behaves like a solid of considerable firmness.
e.   The inhomogeneity of the network of crystals has a very great effect because the fat will start to flow where the bonds are weakest. In a quantitative sense, however, practically nothing is known about this aspect.

As soon as one starts to work a fat its firmness decreases sharply; this is called work softening (see Fig. 19.12C). On keeping, the fat sets again, but not up to the original firmness. Most of the network recovers quickly, but it is complete only after a few hours; a solid structure also forms again, but that takes a longer time. Hence, in a sense, the fat is thixotropic.

As already stated, the fat globules in butter contain a considerable part of the crystalline fat. These crystals therefore cannot or hardly participate in forming a continuous network or a solid structure. In other words, butter is much softer than butter fat (or margarine), with the same amount of solid fat (compare a and b in Fig. 19.12B). Butter is also a water-in-oil emulsion, but the water droplets have only a small effect on the consistency, unless their volume fraction is very great, as in low-fat spreads.

How can we further affect the firmness of the butter, besides varying the temperature, which has an overriding influence (see Fig. 19.13)?

a.   The fat composition has considerable effect, and the milk to be used for butter making may thus be selected, e.g., according to region of origin. Alternatively, cream can be frozen and, in another season, be churned together with fresh cream. Similarly, firm and soft butter can be worked together. The fat composition can be affected via the feed of the cow, but this has not yet become practice.
b.   The method of manufacture has a clear effect, especially the temperature treatment of the cream (and, if need be, the butter grains). Figure 19.13 gives some trends; it appears that the dependence of the firmness on the temperature can also be affected. The temperature treatment of the cream is constrained by certain conditions:
   1.   The souring should be adequate (sufficiently high temperature during sufficient time).
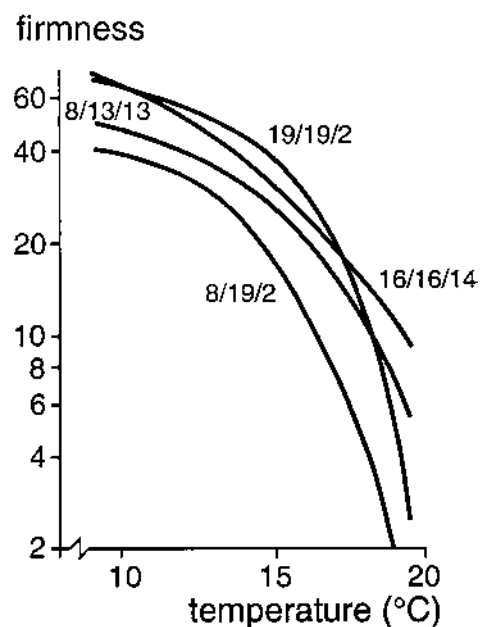
FIGURE 19.13 Firmness (penetrometer, arbitrary units) of butter as a function of the temperature of measurement. Butters from the same cream, but with different cream temperature treatment (indicated on the curves in °C). After H. Mulder, "Zuivelonderzoek", Vol. 2, The Hague, Algemeene Nederlandsche Zuivelbond FNZ, 1947.

2. The cooling of acidified high-fat cream proceeds very slowly due to the low heat transfer coefficient.
3. The churning must proceed satisfactorily and this can only be achieved within a relatively narrow temperature range.
4. The fat content of the buttermilk should not become too high, implying that the liquid fat content should not be too high during churning.

An example is constant temperature, e.g., 13/13/13°C, especially applied to obtain firm butter. (*Note*: The temperature treatment of the cream is usually indicated as a/b/c, where a, b, and c are the successive temperatures.) The fat content in the buttermilk, then, is often high. To make soft butter, cooling in steps is often used; a short deep precooling then is needed to obtain sufficient nucleation, e.g., 8/20/14°C. (This is often called the Alnarp method.) Due to the cooling in steps somewhat less solid fat will result, according to the compound crystal

theory, but the differences are small. On average, the crystals will also be larger and possibly a greater proportion of the solid fat is inside fat globules. A quantitative explanation is lacking. In the continuous butter making from sweet cream (see also Section 19.3), 4/4/12°C often is applied, i.e., after separation the cream is cooled, kept cool in large tanks, raised to the churning temperature by means of a heat exchanger, and churned.

The greater the number of fat globules in butter, the lesser its firmness may be. Very intense and prolonged working can reduce the quantity of globular fat.

c. The keeping conditions have considerable effect. To start with, the butter will always set, occurring faster at a higher temperature (see Fig. 19.14). The cause is formation of solid structures due to changes in the crystallization, such as the rearrangement of compound crystals, polymorphic changes, and Ostwald ripening; these changes proceed more slowly if less liquid fat is available (lower temperature). The setting may continue for a very long time, be it at an ever decreasing rate. It can again become much stronger if the butter is temporarily put to a higher temperature. Then solid fat melts, slowly solidifying again
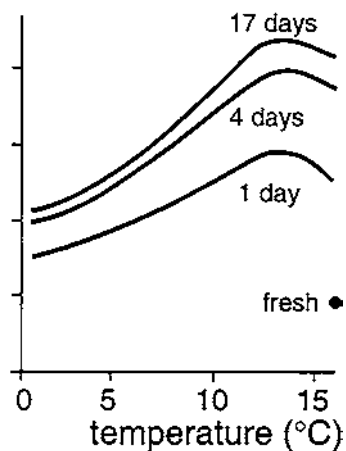
FIGURE 19.14   Effect of temperature and time of storage on the firmness (penetrometer, arbitrary units) of butter, determined at 16°C. The point indicates firmness of the fresh butter made at 16°C. After H. Mulder, ''Zuivelonderzoek'', Vol. 2, The Hague, Algemeene Nederlandsche Zuivelbond FNZ, 1947.

on subsequent cooling, so that a more solid structure can be formed. In that case, the firmness can increase by as much as 70%. In particular, butter made according to the Alnarp method is sensitive to temperature fluctuations. Since these always occur during purchase and use of the butter, the favorable effect of a certain temperature treatment of the cream on the spreadability of the butter may actually be disappointing.

d. Working of the butter causes a strong reduction of its firmness because solid structures are broken (Fig. 19.12C). It is true that the butter sets again, but not up to the original firmness. Hence, to obtain a firm butter, packing should be done immediately after manufacture, while the butter is still very soft (the packing itself involves intensive working); the packed butter then can set fully, especially if it is not stored too cold. If it is desirable to make soft (spreadable) butter, the best policy is to first let the butter set for a considerable time after manufacture and to pack it afterward. To achieve packing, the butter must at first be worked soft in a butter homogenizer.

19.2.3 Cold Storage Defects

To keep butter for a long time, it should be stored at a temperature of, say, $-20°C$. If the butter has been well made, and if the original milk did not contain too many bacteria with thermoresistant lipases, it can keep for a very long time in cold storage. It now deteriorates by autoxidation of the fat, leading to flavor defects after 1 month to 2 years. The factors affecting autoxidation are discussed in Section 2.3.

The keeping quality in cold storage greatly depends on the method of manufacture. Processing variables having an effect are as follows:

a. Contamination with even minute quantities of copper should be strictly prevented.

b. By cooling the milk for some time before its use (e.g., for at least 2 h at 5°C) a part of the copper on the fat globules moves to the plasma; this may restrict the autoxidation. Moreover, the cooling causes a migration of protein to the plasma, and this is precisely the protein that liberates H_2S during heat treatment. Hence, in this way, a cooked flavor after heat treatment can be limited. In many countries most of the milk is already kept cool for a time in the bulk tank on the farm.

c. Heating of milk or cream causes migration of copper from the plasma to the fat globules (see Fig. 19.15A). Pasteurization of the milk should therefore be avoided because more copper is available to migrate in milk than in cream. Even during thermalization not too high a temperature should be selected.
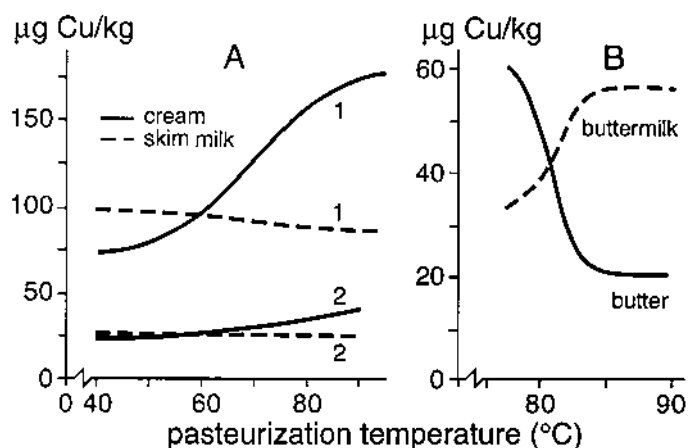
FIGURE 19.15 The partition of copper over the phases as a function of the pasteurization temperature (pasteurizing for 15 s). A. Heating of the milk before skimming; examples of milk with a high (1) and with a low (2) copper content. B. Heating of the cream and estimating the quantities after souring and churning. From H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

    d.   Due to souring of the cream (or the milk) a considerable part (30% to 40%) of the ''added'' copper (i.e., copper entered by contamination) moves to the fat globules. Because of this, butter from sour cream is much more affected by autoxidation than that from sweet cream. But sweet cream butter is not aromatic.

    e.   With reference to the migrations mentioned in items c and d, it is important to adjust the fat content of the cream to a high level because this causes a lower copper content in the butter.

    f.   Heating of the cream largely prevents the migration during souring mentioned in item d (see Fig. 19.15B). In all probability, Cu becomes bound to low-molar mass sulfides, especially H_2S, formed by the heat treatment. This causes a strong reduction of the autoxidation in sour cream butter. Therefore, it would be desirable to pasteurize the cream very intensely, but then much H_2S is formed, yielding butter with a gassy or cooked flavor. Although this flavor defect decreases slightly during storage, it is objectionable. The pasteurizing conditions should thus be optimized (not too high, not too low); of course, the smaller the spread in holding time, the better.

    g.   Adding salt to sour cream butter considerably accelerates the autoxi-

dation. In sweet-cream butter a high salt content has an oxidation-diminishing effect.

h. The lower the storage temperature, the longer the keeping quality.

19.3 CULTURED BUTTER FROM SWEET CREAM

It is often a problem to dispose of sour cream buttermilk because it has a very short shelf-life and the demand as a beverage often is small. Moreover, it cannot be pasteurized. Sweet cream buttermilk can be processed much more easily. On the other hand, several markets prefer an aromatic butter, which has to contain acid (lactic acid) and aroma substances (mainly diacetyl). It has indeed been tried to churn sweet cream and to add starter to the butter granules afterward to work it into the butter, but the result is disappointing, i.e., the flavor remains almost equal to that of sweet butter. This is not surprising because the souring and the diacetyl production in the butter can hardly occur if the moisture has been well dispersed (see Fig. 19.9); the pH also remains high.

NIZO has developed an alternative manufacturing process. Sweet butter grains are worked together with a very aromatic starter and with a concentrated starter permeate, essentially a lactic acid solution. A flow sheet of the butter making is shown in Figure 19.16; churning and working can proceed in a churn-and-worker or in a continuous butter-making machine. The initial water content (i.e., after the first working) should be low so as not to exceed the 16% limit afterward. In preparing the starter permeate a partly delactosed whey is soured by means of *Lactobacillus helveticus*; then the liquid is purified by ultrafiltration, and the permeate concentrated by evaporation. The lactic acid content of the permeate then amounts to about 16% (standard acidity degree 1800°N).

At first a normal aromatic starter was worked in, apart from the starter permeate. This had the disadvantage that the aroma must develop after the butter making and that the aroma formation depends too much on the conditions; hardly any aroma is produced if the butter is immediately transferred to cold storage, whereas at room temperature the aroma production can be excessive if the butter has a not-very-fine moisture dispersion. Therefore, nowadays a very aromatic starter is grown in evaporated skim milk (starter I in Fig. 19.16); it is mixed with the starter permeate and subsequently aerated. In this way sufficient diacetyl is formed to give the butter a quantity of >1 mg/kg from the very beginning; the bacteria are killed by the high lactic acid content, so that no reduction of diacetyl can occur. Starter II contributes some other flavor components and can cause a continued formation of diacetyl; the concentration of diacetyl in the butter eventually is 1.5–2.5 mg/kg.

It will be obvious that moisture droplets will occur in the butter that more or less differ in composition: sweet buttermilk, starter, starter permeate with starter. Some compounds can migrate, e.g., water and lactic acid (both slowly),
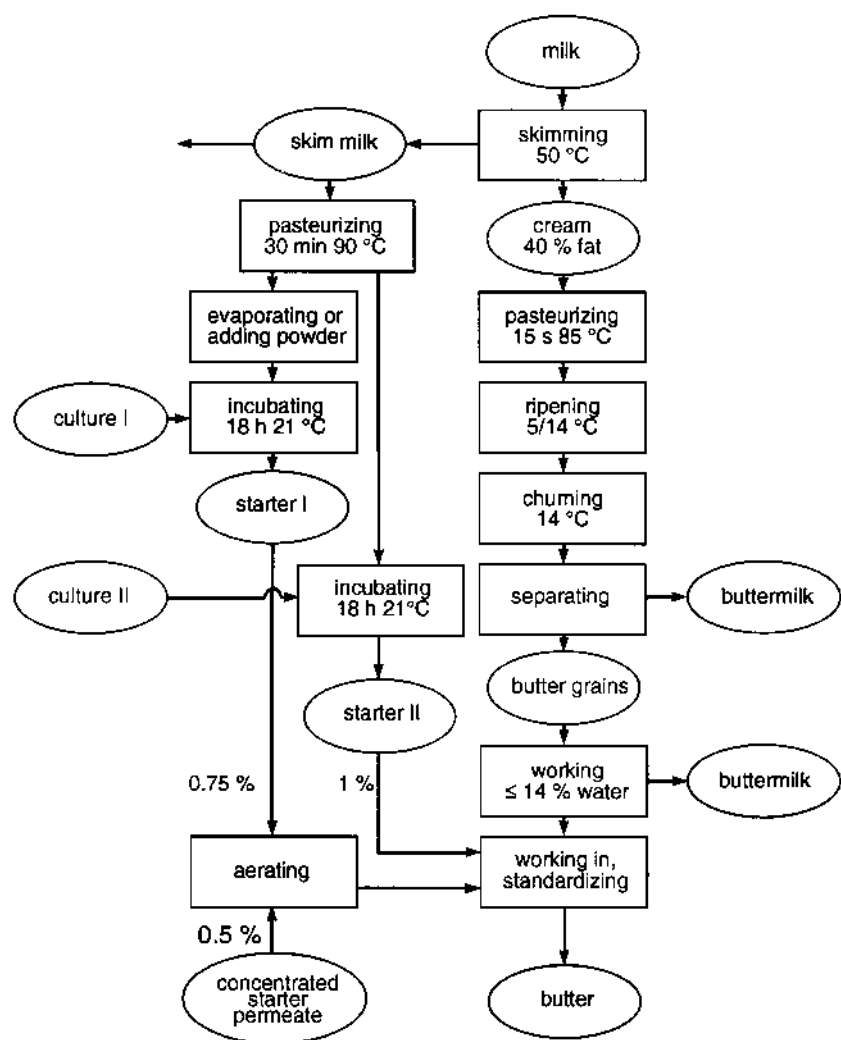
Figure 19.16 Example of the manufacture of aromatic butter according to the NIZO method.

and especially diacetyl, which is fairly fat-soluble (the partition coefficient water/oil roughly equals unity).

The processing is rather more complicated than for traditional butter making, but that need not be a problem in large centralized plants. Moreover, the following may be important differences with the traditional butter making:

a.   Sweet cream buttermilk is obtained (originally the main purpose of the process).
b.   Less starter is needed.
c.   The desired starters are very phage-sensitive (especially starter I). Contamination by bacteriophages thus should be prevented.
d.   The solids-not-fat content of the butter increases slightly, hence the yield.
e.   The aromatic flavor of cultured butter is more pronounced.
f.   The copper content of the butter can be much lower (see Section 19.2.3, item d). Not surprisingly, the butter is very stable to autoxidation.
g.   The quantity of free fatty acids in the butter can be lower. In the cream a partition equilibrium exists between free fatty acids in the fat and in the plasma. The lower the pH, the less the dissociation of the fatty acids ($pK_a \approx 4.8$); hence, the higher their solubility in the fat and the lower in the water. Moreover, it appears that the acidity of the fat is somewhat increased by churning at low pH (explanation unknown). Hence, butter from sour cream will contain a larger amount of free fatty acids and will more readily attain a soapy-rancid flavor.
h.   Since during cream ripening the souring need not be considered, one is more free to select the best temperature treatment to control butter consistency. In general, there are more degrees of freedom in the way of processing.
i.   The sweet cream can more easily be pumped and passed through a heat exchanger.

Apart from item c, these are all of advantage to the manufacturer.

19.4   HIGH-FAT PRODUCTS

Besides butter there are other high-fat products in which fat is the continuous phase. Such products are made for various reasons.

Traditionally, butter was ''melted down'' to increase its keeping quality, i.e., after heating the butter, the formed butter oil was separated. The rendered butter thus obtained had a long shelf life. Nowadays, this product is designated anhydrous milk fat; it may be used as such in the kitchen because, contrary to butter, it allows heating at a high temperature. In a country like India where the temperature may be too high for butter making, *ghee* is made from buffalo's milk. This milk has fairly large-sized fat globules, which at a somewhat higher temperature cream sufficiently fast (without agglutinating) to yield a cream layer. The cream obtained is subsequently heated over an open fire until the water has boiled off. Precipitated dry matter substances are removed by decanting.

The separated fat can be modified and/or fractionated in various ways. The main aim may be to change the crystallization behavior of the fat. The modified fat can be used in recombined butter, in chocolate (as a partial substitute for cocoa butter), in bakery products (such processes as kneading of dough and paste, and beating in of air require a constant melting behavior and often a high final melting point), in recombined cheese (restricting oiling-off), or in instant milk powder (liquid fat yields better instant properties).

From milk fat and skim milk recombined butter can be made. The purpose may be to enhance the value of poor-quality cream or to obtain a product with other properties, e.g., another firmness (spreadability), a higher water content, or a higher content of polyunsaturated fatty acids. For example, several kinds of spread are made, especially low-fat spreads. To achieve this, blends of milk fat and vegetable oil can be used. Alternatively, a vegetable oil (e.g., 25% to 30% refined soybean oil) can be worked into butter to obtain a spreadable mixture, such as the Swedish Bregott.

Some processes and products are now briefly discussed.

19.4.1   Anhydrous Milk Fat

The most important general requirement is that the fat be very pure and stable to autoxidation. To secure this, good-quality fresh milk should be used. Contamination by traces of copper is highly detrimental. The water content should not exceed 0.1%, because otherwise moisture droplets may form at low temperature. If the water content is higher (up to 0.4%), the product is usually designated ''butter oil.''

There are numerous manufacturing processes. As a rule, one starts from butter. Alternatively, one can make high-fat cream (by centrifuging twice) and accomplish a phase inversion in it: If a very concentrated o/w emulsion is destabilized, a w/o emulsion is usually formed. To achieve this, fat cream can be passed through an agitator, a special pump, or even a homogenizer; often, the phase inversion occurs easier if the cream first is subjected to ''washing,'' i.e., diluting it with water and reseparating. If cream with 82% fat is passed through a scraped-surface heat exchanger while being cooled sufficiently for fat crystallization to occur, then butter is formed. Examples are the Alpha process and the Meleshin process. Sweet cream Alpha butter can be very durable.

In the manufacture of anhydrous milk fat, the cream destabilization usually occurs at high temperature and a butter oil containing plasma droplets then is obtained; often, a more or less complete separation into two layers immediately sets in. This can also be achieved by melting of butter. Subsequently, a separation has to take place by decanting or, in common practice, by centrifuging; for this purpose a suitable separator is needed. (Generally the plasma is, once again, passed through a normal cream separator, yielding a little cream.) The fat ob-

tained in this way is very pure; if the temperature during the melting and separating has not been too high, it is almost free from polar lipids.

Another method of working is based on evaporation of water by heat treatment: from butter, from fat cream, or from an intermediate product, e.g., butter grains; alternatively, washed cream can be used. When the evaporation is done in an open vat, i.e., at atmospheric pressure, the temperature becomes somewhat high, up to 120°C; the product obtained is ghee. Furthermore, cream or butter can be ''dried'' in a vacuum evaporator or in a spray drier. In all cases, the nonfat solids are left dispersed in the fat. These can be removed by decanting, filtering, or centrifugation. Due to the higher temperatures and the strong lowering of the water activity, the polar lipids, especially the phospholipids, are altered to such an extent as to partly dissolve in the fat. In this condition phospholipids have an antioxidant activity, which is beneficial for the keeping quality. Genuine ghee, especially ghee made from sour cream, contains numerous flavor compounds originating from phospholipids, proteins, and triglycerides (e.g., methyl ketones from keto fatty acids). Ghee is often appreciated for its flavor.

Milk fat made by one of the above processes usually contains about 0.4% water. On cooling, droplets are formed (the solubility of water in milk fat is 0.1, 0.2, and 0.4% at 10, 40, and 90°C, respectively) and the product can spoil rapidly. Therefore, vacuum drying is generally applied, e.g., at 40°C and 2 kPa (= 0.02 bar). It causes a decrease of the water content to below 0.1%. Also the oxygen content decreases significantly. A product made in this way may keep for some years if made from milk without any beginning autoxidation, if stored in isolation from air and light, and if copper contamination has been rigorously prevented.

19.4.2 Modification of Milk Fat

The most widely used modification is *fractionation* by means of crystallization. After solidification of milk fat at a certain temperature in such a way as to form fairly large crystals, the fat can mechanically be separated into a solid and a liquid portion. The purpose is to obtain fractions with different melting behavior. The composition is also altered in another respect since fat-soluble components, like carotenoids, vitamins, and flavors, become concentrated in the liquid fraction. Accordingly, it remains to be seen as to whether a ''solid'' fraction may still be considered milk fat.

The success of a single fractionation is less than expected. The separation is incomplete (see Fig. 19.17A) because the network of fairly small crystals readily retains liquid fat. To be sure, one can attempt to form large crystals by cooling the fat very slowly. This may cause formation of spherulites. Spherulites are sphere-shaped crystals, but they are made up of a great number of ramified radial needles, between which, again, liquid fat is held. A far better fractionation can be achieved by crystallization of the fat from acetone, but this is an expensive
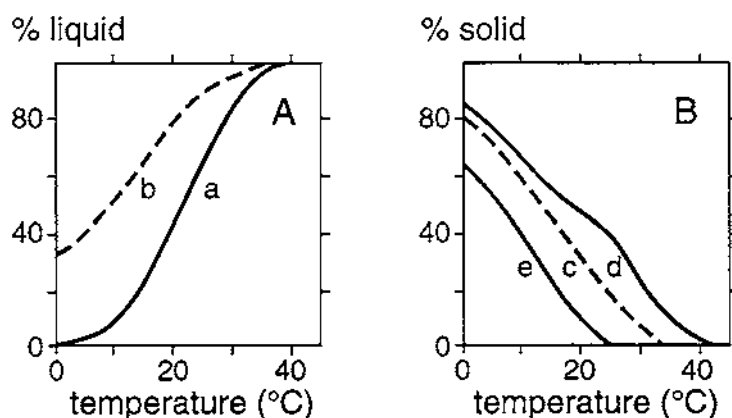
FIGURE 19.17 Examples of fractionation of milk fat. (A) Amount of liquid fat that could be separated as a function of the solidification temperature applied (curve a), in comparison with the amount of liquid fat actually present in the fat (b). (B) The percentage of solid fat as a function of the melting temperature applied. The ''solid'' (d) and the ''liquid'' (e) fractions, obtained by fractionation at 25°C, are compared to the original fat (c). (From various sources.)

method; moreover, use of the product obtained in foods may not be allowed by the public health authorities.

Furthermore, the difference in melting curve of the various fractions is disappointing (Fig. 19.17B). This must be ascribed at least partly to the strong tendency of milk fat to form compound crystals. Fractionation in steps, combined with optimization of the separation method, can yield good results, although it takes a long time (some days). Milk fat fractions are frequently used in practice, especially to make butter more spreadable. It has also been tried to make recombined butter with a firmness that is not greatly temperature-dependent. Using blends of high-melting and low-melting portions may score some success but is expensive.

Milk fat can also be modified chemically, but the products can no longer be called milk fat. *Hydrogenation* (by using H_2 and a catalyst at high temperature) decreases the number of double bonds and thereby increases the high-melting proportion of a fat; this is why the process is often called hardening. It also causes several other changes, such as displacement of remaining double bonds and cis–trans isomerization. Hydrogenation can be applied to make cocoa butter replacers from milk fat. Interesterification causes the distribution of the fatty acid residues over the positions of the triglyceride molecules to become increasingly random. It can be achieved by heating the fat in the presence of a catalyst, like sodium

methoxide. (At very high temperature—say, 150°C—it also occurs without a catalyst being present.) After interesterification, the melting range of milk fat is shifted to higher temperature. Similar effects occur on cis-trans isomerization. None of these chemical modifications is applied to milk fat on a commercial scale.

19.4.3 Recombined Butter

Milk fat and skim milk can be recombined to yield a butter-like product. Manufacturing process and physical structure of the product then are practically identical to those of margarine. The difference is in the composition.

A disadvantage is that recombined butter is firmer than natural butter of the same fat composition; the texture is also slightly different. This is largely due to the product not containing fat globules, so that all fat crystals can participate in networks and solid structures. Accordingly, the firmness has to be adjusted in a different way, mainly via the fat composition. The final melting point of the fat should be below body temperature because the butter should fully melt in the mouth. If temperature fluctuates widely a coarse texture readily develops, due to the fat crystals becoming very large.

A simplified outline of the manufacturing process is given in Figure 19.18. It is largely self-evident. In the production of margarine a good deal precedes in the way of treatment of the fat, including degumming, alkali refining, bleaching, deodorizing, and partial hydrogenation.

The treatment of the aqueous phase mainly serves to attain a good flavor and to improve the keeping quality (preventing spoilage by microorganisms, lipase, etc.). Usually, in the production of recombined butter, hardly any additives are used, whereas margarine may contain an antioxidant with or without synergist, coloring matter, added vitamins A and D, fat-soluble flavoring agents, and an emulsifier.

Emulsification serves to obtain a fairly homogeneous mixture of constant composition. The water-in-oil (w/o) emulsion is not stable and the liquid must be agitated at all times, until fat crystals have been formed. The crystals stabilize the moisture droplets by adsorbing onto the interface (Pickering stabilization). The emulsifiers (in margarine, e.g., monoglycerides and soy bean lecithin) serve other purposes. Monoglycerides seem to prevent the fat crystals from flocculating so strongly into a network that they can insufficiently adsorb onto the moisture droplets. Moreover, the emulsifier plays a twofold role during the heating of the product in the frying pan. Due to melting of the fat the moisture droplets become unstable, and if they now quickly flow together into large droplets these can start to splash very annoyingly when reaching the boiling point. The emulsifier slows down such coalescence. Furthermore, lecithin contributes to the typical aroma during frying. Therefore, in the case of recombined butter it has its advantages
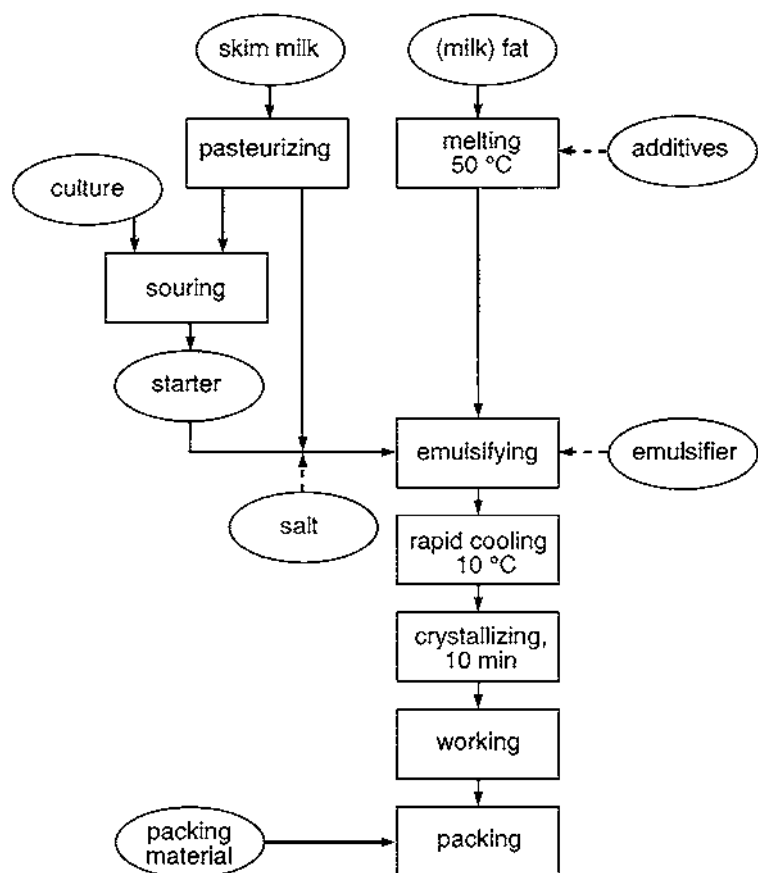
FIGURE 19.18 Example of the manufacture of recombined butter (or margarine).

to displace a part of the skim milk by sweet cream buttermilk; in this way the composition also is closer to that of natural butter.

The cooling is usually performed in a scraped-surface heat exchanger; otherwise the heat transfer would proceed far too slowly, causing the formation of overly large crystals. Due to the scraping and stirring the moisture droplets, which during emulsification may still be up to 1 mm in size, are divided into much smaller ones. A further reduction occurs by the working that consists of forcing the mass through a small opening (valve) or a perforated plate (see Section 19.1.4).

The working also serves to destroy solid structures of fat crystals. These structures should then indeed already have been formed, which implies that by far most of the crystallization should have taken place. This is achieved by the passage through a ''crystallization tube'' (B unit) before the working. However, milk fat crystallizes very slowly and, accordingly, it is mostly impossible to achieve a fairly complete crystallization in one processing step (as is common in margarine manufacture). Consequently, the butter can still set considerably after manufacture. During crystallization one often has to cool again. If, for instance, 20% of the fat crystallizes, then the heat of crystallization suffices to increase the blend temperature by 8°C.

19.4.4 Butter Products with a Low Fat Content

Low-fat butter products have long been in existence (from the early 1940s), but they have received renewed interest following low-fat margarine. These ''spreads'' have a water content of, say, 40%. There are also spreads that partly consist of fat extraneous to milk fat and that are held to be better for health than butter by some people.

Production of low-fat butter cannot be achieved by churning because the formation of a product with a discontinuous aqueous phase fails. Most surface-active substances in cream are water-soluble, and this largely prevents the formation of a w/o emulsion. (Only at a very high fat content and in the presence of fat crystals is a phase inversion from cream to butter possible. Melting away of the crystals destabilizes any formed w/o emulsion and results in a continuous aqueous layer.)

In the production of the butter product, a manufacturing process comparable to that in Figure 19.18 should be applied; a suitable (i.e., oil-soluble) emulsifier should be added. It may be a problem to make the moisture droplets sufficiently small and to prevent them from coalescing in the product, especially at a somewhat higher temperature. Therefore a gelling agent (e.g., gelatin) may be added. It causes the droplets to become more or less solid, unable to coalesce. At the least, a thickening agent should be added to the aqueous phase. This may be a protein mixture, like serum protein that has been coagulated to yield aggregates of about 1 μm in size. The agent improves flavor and mouth-feel of the product, which tends to be similar to butter. Flavor substances as well as aroma-forming starter bacteria may be added. All the same, there is no common appreciation of the flavor of such a ''butter.'' One of the aspects involved may be that the product contains less crystalline fat than butter does and therefore feels less cool in the mouth.

Spreadability is generally not a problem. However, if the butter product contains excess liquid fat it may become too soft and at room temperature it may not retain its shape and show oiling-off. A product with a low number of fat

crystals might have large moisture droplets, which can easily cause microbial spoilage (Section 19.1.4). A preservative can then be added.

An alternative technique for the manufacture of low-fat butter is to incorporate a fairly viscous aqueous compound (e.g., a pasteurized caseinate solution) into natural butter. Some authors say that in this way a bicontinuous system is formed. In any case, a preservative is needed, and this certainly holds for spreads of the o/w type, essentially fat cream that has been transformed into a spreadable product by the use of thickening and gelling agents. Naturally, the latter products behave differently from natural butter. For example, the included moisture will migrate into the bread onto which it is spread. Obviously, making a good-quality low-fat butter is far from easy.

SUGGESTED LITERATURE

- There is very little recent literature about the manufacture and properties of butter.
- General and comprehensive information can be found in:

  H. Pointurier and J. Adda, *Beurrerie Industrielle: Science et Technique de la Fabrication du Beurre*, La Maison Rustique, Paris, 1969.

- Physicochemical aspects of churning, butter, and anhydrous milk fat are treated in:

  H. Mulder and P. Walstra, *The Milk Fat Globule*, Pudoc, Wageningen, 1974.

- Additional information can be found in:

  P. F. Fox, ed., *Advanced Dairy Chemistry*, Vol. 2, *Lipids*, 2nd ed., Chapman and Hall, London, 1995, Chapters 4 (Physical chemistry of milk fat globules) and 5 (Crystallization and rheological properties of milk fat).

20

Fermented Milks

20.1 GENERAL ASPECTS

20.1.1 Preservation

The fermenting of milk is a fairly simple, cheap, and safe way to preserve milk. In regions with high-quality milking and milk collection system, a high level of technological know-how to preserve raw milk, and good transportation and distribution facilities, the need for fermentation as a preservation method does not exist. In regions where such facilities are lacking, the fermenting of milk as a means of preservation has kept its original importance. The lactic acid bacteria alter the conditions in the milk in such a way that most undesirable organisms, including pathogens, cannot grow or even die. These conditions include a low pH (4.6–4.0), which also helps in maintaining a low pH in the stomach after consuming the milk; growth inhibition by undissociated acids (e.g., lactic acid) and by other metabolites such as H_2O_2 and compounds with an antibiotic activity; a low redox potential; consumption by the lactic acid bacteria of compounds that are vital for the growth of other organisms. Appropriate pasteurization of the raw milk kills any pathogens that may survive the fermentation.

Microbial, chemical, and physical spoilage of fermented milk products can occur. Yeasts and molds may keep growing at a pH below 3.8. Accordingly, these are by far the most important sources for microbial defects. Pasteurizing the milk and preventing its recontamination are thus required. The growth of aerobic yeasts and molds in recontaminated products is determined by the extent of contamination, the temperature and time of storage, the amount of air in the package, and the air permeability of the packing material. Incidentally, in certain

types of sour milk products yeasts, and sometimes also molds, are part of the essential flora. When the products are kept for a long time or when they are stored at a high temperature, the ongoing action of enzymes of the starter bacteria can also cause defects, such as bitter, acid, and cheesy flavors.

An excessive acid production in the milk leads to an objectionable acid flavor. At equal titratable acidities, the pH of concentrated milk products is higher, which causes the effect of an excessive acid production on the flavor to be less readily perceptible. In former days, when appropriate refrigerating systems were lacking, advantage was taken of this phenomenon, e.g., by concentrating milk for yogurt making. A satisfactory process control and an adequate refrigeration during storage and distribution are the common industrial tools to prevent a low pH. Contamination by metals like copper and iron and exposure to light may have adverse effects on the flavor of the fermented products. The packing material can also cause off-flavors.

Physical deterioration especially manifests itself in wheying off. Factors involved include:

a. *Pretreatment of the milk*. Some process steps that increase the viscosity (pasteurization, homogenization) also decrease the risk of wheying off.
b. *Composition of the milk*. A lower casein content increases the risk of the defect.
c. *Incubation temperature*. The higher it is, the greater the tendency for whey separation.
d. *The pH of the product*. A high as well as a low pH enhances wheying off (see Fig. 21.5). Clearly, the starter should be sufficiently vigorous, and the product should be cold-stored.
e. *Mechanical treatments* of the fermented milk, such as stirring and pumping, should be performed cautiously, especially before pH 5 is reached.
f. *Presence of air cells and gas cells*. Beating in of air during manufacture of the product enhances wheying off, and so does gas production by coliforms, yeasts, etc. Excessive production of CO_2 by starter bacteria may also cause it and it may follow from a change of CO_2 from the dissolved into the gaseous state caused by an increase in temperature. Therefore, for instance, buttermilk is degassed to some extent before packing.

20.1.2   Nutritive Value

Studies on the nutritive value of fermented milks predominantly deal with yogurt and are mainly carried out on animals. Determining the significance of the observed effects for human health is not easy. Following are the most important aspects when a fermented milk product is compared with plain milk.

20.1.2.1 Composition

a. *Lactose content*. Fermentation decreases the lactose content but is not continued to such a low pH that any further sugar breakdown is impossible because the resulting product would become too acidic. At a lactic acid content of, say, 0.9% the fermentation is often slowed down by cooling. About 20% of the lactose in the milk has then been split, if both glucose and galactose are fermented. In yogurt twice as much lactose is split since most of the yogurt bacteria do not decompose galactose.

b. *Vitamin content*. Lactic acid bacteria often require certain B vitamins for growth, and can produce other vitamins. Accordingly, the properties of the culture involved largely determine the extent to which the concentrations of vitamins in the fermented milk differ from those in the original milk. In yogurt the level of most of the vitamins is somewhat reduced; the folic acid content may be increased but the utilizability by humans of the folic acid thus formed is not certain. The vitamin contents in fermented products are also affected by the storage conditions and especially by the pretreatment of the milk. For instance, heat treatment of milk results in a decrease of vitamins B_1, B_{12}, C, and folic acid (see Section 6.2).

c. Other changes due to bacterial action are nutritionally insignificant.

d. Composition can be changed by such process steps as standardization and ultrafiltration, and by addition of skim milk powder, caseinates, stabilizers, flavorings, or fruit pulp.

20.1.2.2 Nutritional Aspects

a. *Edible energy*. The fermentation process per se does not cause a substantial change of the energy content of milk. The conversion of lactose to lactic acid reduces the energy value by only a small percentage.

b. *Digestibility*.
Protein and fat. The digestibility may be improved by a slight predigestion of the compounds by enzymes of the lactic acid bacteria. People with a weakened intestinal function may take advantage of the predigestion, but healthy people digest these compounds efficiently. In the stomach, the protein in fermented milks coagulates into finer particles than in plain milk, which may increase digestibility. The gastric juice of babies contains little acid and, accordingly, sometimes (dextrorotatory) lactic acid is added to baby formulas.
Lactose. Lactose-intolerant users digest a sour milk product like yogurt much better than plain milk. The lowered lactose content plays a part.

In addition, factors must exist that cause easier digestion of lactose. The lactase activity of the yogurt bacteria as well as the stimulation of the lactase activity of the intestinal mucosa by yogurt have been held responsible. Alternatively, the depletion of the stomach contents into the duodenum may be retarded when fermented milks are consumed; thereby, the contact time of lactose hydrolyzing enzymes with the substrate in the stomach would be extended, resulting in a better digestion of lactose.

c. *pH adjustment*. The consumption of fermented milks causes a smaller increase of the pH of the stomach contents and thereby diminishes the risk of passage of pathogens. This is of particular importance for people suffering from a weakened secretion of gastric juice, e.g., many elderly people and babies.

d. *Antimicrobial action*. Lactic acid bacteria can form antibiotic compounds that injure pathogens in vitro. The in vivo significance of these compounds in suppressing gastroenteritis is not quite clear.

e. *Absorption of minerals*. Due to the low pH of fermented milks, some minerals are better soluble than in plain milk; it is sometimes assumed that a better absorption of minerals is thus to be expected. However, the absorption of various elements, especially that of magnesium and zinc, is enhanced by lactose. The lactose content decreases during fermentation, causing the net absorption from sour milk to be lower. Animal tests with yogurt have confirmed the effect; the absorption of phosphorus, which is less affected by lactose, proved to be somewhat increased. Clearly, as far as the uptake of minerals is concerned, the fermentation of milk offers no distinct nutritional advantages.

f. *Some additional positive and negative effects*.

*   *Intestinal flora*. The consumption of living lactic acid bacteria through fermented milk is supposed to result in the implantation of a favorable flora of lactic acid bacteria in the large intestine; the flora might repress pathogens. The most probable effects result from bacteria that do not only survive the action of gastric juice in the gastrointestinal tract but can also colonize in the intestine, such as strains of the intestinal bacteria *Lactobacillus acidophilus, L. salivarius*, and *Bifidobacterium bifidum*. When yogurt is eaten frequently, the common yogurt bacteria survive the transport through the gastrointestinal tract but do not colonize. Thus far investigations have not permitted the conclusion that effects for humans are favorable.

*   *Cholesterol level*. Some animal tests suggest that consuming fer-

mented milk might contribute to a decreased cholesterol content of the blood and, accordingly, could reduce the risk of approaching heart and vascular diseases. However, even if this is true, the effect would be small. Consumption of fermented milk could further contribute to an increased resistance to pathogens by activating the immune system and to a decreased risk of colon cancer. However, positive effects for humans have not yet been demonstrated.

- *Dental caries*. Fermented milks have not been shown to cause caries due to damage to the enamel at low pH. The lactic acid bacteria of the mouth flora form no sticky dextrans from lactose (they form these from sucrose) and they consequently cause no dental plaque. Obviously, the saliva has adequate counteracting activity to prevent dental caries.
- *Cataract*. Supposedly, the consumption of yogurt can cause this eye disorder. Rats exclusively fed with yogurt (made of concentrated milk) went blind because of the accumulation of galactitol in the eye lens. However, unlike the rat, humans can readily convert galactose to glucose; therefore, the galactose content of the blood does not increase and no galactitol is formed.
- *Lactic acid type*. The type of lactic acid formed has physiological significance. Two stereoisomers of lactic acid exist: dextrorotatory L(+) lactic acid and levorotatory D(−) lactic acid. L(+) lactic acid can readily be metabolized in the body, but D(−) at a slower rate. The latter acid is partly removed from the body through the urine. In traditional yogurt some 40% to 60% of the lactic acid is levorotatory and is formed by *Lactobacillus delbrueckii* ssp. *bulgaricus*. Ingesting excessive quantities of D(−) lactic acid may cause acidosis, resulting in some tissue injury. Young infants are more susceptible to acidosis than adults. Before 1974, the World Health Organization recommended a daily intake of D(−) lactate of less than 100 mg per kilogram of body weight. This limiting value was practically irrelevant to adults, since a 75-kg body weight would allow digestion of 1.5 liters yogurt per day. The recommendation has been withdrawn; nevertheless, not too much D(−) lactic acid should be fed to infants younger than 3 months.

20.2 VARIOUS TYPES

Fermented milks can be subdivided according to a number of variables, such as type of fermentative process, fat content, concentration of the milk, withdrawal of whey, and use of milk from various species.

20.2.1   Type of Fermentation

Within this grouping there are:
1. Products with a rather pure lactic fermentation. The fermentation is by:
   a. *Mesophilic starters*, consisting of *Lactococcus lactis* ssp. *cremoris* or ssp. *lactis, Leuconostoc cremoris/lactis* and/or *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*. Examples are sour milk, buttermilk and related products, sour cream, ymer, langfil, and viili.

      LANGFIL (long milk, ropy milk) is a representative of highly viscous, ropy sour milk products, manufactured in northern Europe. A DL- or a D-starter is used. Polysaccharide-producing strains of *Lactococcus lactis* ssp. *cremoris* are responsible for the high viscosity. A relatively low incubation temperature (about 18°C) enhances the polysaccharide production. The high viscosity of the product prohibits bottle filling and, accordingly, the fermentation occurs in the package.

      VIILI, a Finnish product, is made of pasteurized nonhomogenized milk. A polysaccharide-producing starter is applied, comparable to that used for langfil; the milk is incubated at 18–19°C for 18–20 h. A mold, *Geotrichum candidum*, is also added to the milk. A gravity-separated cream layer is formed, on which the mold creates a velvety layer and causes some hydrolysis of fat. The latter factor may increase the contribution of the lactic acid bacteria to lipolysis (see Section 23.4). The product is hermetically packed. The mold stops growing after O_2 has been completely consumed. Most of the CO_2 formed dissolves in the product, leading to a slight underpressure in the package.

   b. *Thermophilic starters*, composed of:
      - A protocooperative flora of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, used in yogurt (see Section 20.3.1).
      - A pure culture of *Lactobacillus acidophilus*, used for the manufacture of acidophilus milk; or a flora of this organism and/or *Bifidobacterium bifidum* combined with the yogurt bacteria, used for the manufacture of yogurt-like products.

        ACIDOPHILUS MILK owes its existence to its supposed therapeutic value (see Section 20.1.2). *L. acidophilus* is not a natural representative of the milk flora. It grows slowly in milk and, hence, contamination during the manufacture of acidophilus milk must be avoided. Sterilized milk is inoculated with a large amount of starter (2% to 5%) and incubated at about 38°C for 18–24 h. The product is cooled to 4°C and distributed soon. This is because *L. acidophilus* is fairly acid-tolerant, due to which the lactic acid content of the milk can become high, i.e., some 1% to 2% if stored at an insufficiently low temperature; the flavor of the milk becomes sharp and the number of living bacterial cells de-

creases quickly. The latter problem can be overcome by blending plain milk with a deep-frozen concentrated culture of the starter, and by keeping the mixture at a low temperature (say, 4°C), which prevents the milk from souring.

YOGURT-LIKE PRODUCTS may contain *L. acidophilus* and/or *B. bifidum* in addition to *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. Alternatively, *S. thermophilus* can be combined with *L. acidophilus*, or with *B. bifidum*, or with both types of bacteria. The resulting products are designated as Bioghurt, Bifighurt, and Biogarde, respectively. Bioghurt is also manufactured by using *Lactococcus lactis* ssp. *taette* (isolated from the Norwegian ''taette'') and *L. acidophilus*. The latter bacteria may have a stimulating effect on the growth of each other; this is called protocooperation.

2. Fermented milks combining a lactic fermentation with alcohol production, e.g., kefir and kumiss (see below).

20.2.2 Fat Content

Considerable differences in fat content can exist between various types of fermented milks as well as within one type. Examples are sour milk, buttermilk and related products, and sour cream.

SOUR MILK is prepared by acid production in whole or separated milk at 20°C by using a D starter. The standards for the fat content vary widely and this holds also for the lactic acid content (0.5% to 1.5%).

BUTTERMILK derives from the churning of cultured cream in the manufacture of butter. Related products (cultured buttermilk or cultured skim milk) are made by souring skim milk at 20°C using an aromatic L or DL starter. Sometimes, the milk used for the manufacture of cultured buttermilk is required to have a minimum fat content of, say, 0.4%. This is because the flavor becomes too acid at lower fat contents. The milk is preheated (e.g., 20 s at 80–85°C) to increase the viscosity of the cultured buttermilk. After having attained the desirable acidity for the viscosity and the flavor, the milk is stirred until it is smooth, degassed, cooled, and stored at about 4°C.

Real or churned buttermilk has a more characteristic flavor than cultured skim milk, probably due to the higher level of membrane compounds of fat globules, especially phospholipids. The higher the fat content of the cream, the higher the phospholipid content of the resulting buttermilk. The difference in composition renders cultured skim milk far less susceptible to oxidized flavor (see also Section 2.3). Churned buttermilk, especially if derived from high-fat cream, readily shows flavor defects and thereby becomes unacceptable. Vitamin C can be added to retard lipid oxidation. At present, buttermilk is sometimes manufactured by churning sour milk; the phospholipid content will be only a little higher

than in cultured skim milk, causing the product to have a longer shelf life than traditional buttermilk.

SOUR CREAM is high-pasteurized, 18% to 20% fat cream, homogenized (preferably at a low temperature, to promote formation of homogenization clusters; see Section 8.7), and is inoculated with an aromatic starter and incubated at 20°C. During the acid production the homogenization clusters flocculate, resulting in a highly viscous cream. To increase the firmness some rennet and/or a thickening agent are sometimes added to the sweet cream. When the pH has reached 4.5 the cream is cooled during gentle stirring and packed. Alternatively, souring in the package may be applied.

20.2.3   Concentration of the Milk

The milk used for making concentrated yogurt is evaporated. Excessive acid production then is less detrimental to the flavor due to the higher buffering capacity of a concentrated product.

20.2.4   Withdrawal of Whey

This is another way to prepare concentrated milks. An example is the manufacture of ymer.

YMER is a Danish soured milk drink. High-pasteurized milk is acidified to pH 4.6 by using an aromatic starter; the sour milk is gradually heated to 35°C, and some syneresis occurs. The CO_2 formed causes the curd to float. The whey is drawn off, homogenized cream is added to the curd, and the mixture is stirred, cooled, and packed. Ymer contains 11.5% fat-free dry matter, 6.5% protein, and 3% fat (see also Table 25.2). It is a high-protein and relatively low-calorie product, with a fairly thick but pourable consistency. Alternatively, ymer is made by direct fermentation of ultrafiltered milk; the product is stirred and packed after fermentation. Various other concentrated fermented milks are made by applying ultrafiltration processes.

20.2.5   Milk of Various Animal Species

Most fermented milks are made of cows' milk. For some products milk of other species is applied, especially ewes', goats', and mares' milk. Well-known products are kefir and kumiss. There is also Greek style yogurt, i.e., a type of concentrated yogurt. It is made of ewes' milk and therefore has a high fat content (see Table 1.5).

KEFIR is made of ewes', goats' or cows' milk. During the fermentation lactic acid and alcohol are produced. Originally, the milk drink was made in Russia and southwestern Asia. It is now being made in various countries on an industrial scale by using cows' milk.

The microflora of kefir is variable. Lactococci (*Lactococcus lactis* ssp. *lactis*, ssp. *cremoris*, and biovar. *diacetylactis*), leuconostocs (*L. lactis, L. cremoris*), and lactobacilli (*L. brevis, L. kefir*, sometimes also *L. delbrueckii* ssp. *bulgaricus, L. acidophilus*) can form lactic acid, while yeasts, including *Candida, Kluyveromyces*, and *Saccharomyces* species, produce alcohol. Kefir of a satisfactory quality is believed to contain acetic acid bacteria also. Typically, the organisms involved in the cultured product are present in structures (grains). During fermentation of the milk the grains grow due to coagulation of protein, while they become connected by means of a formed polysaccharide (''kefiran'').

Kefir is a cream-like, sparkling, acid milk drink. Its lactic acid content is 0.7% to 1% and its alcohol content ranges from 0.05% to 1%, but is rarely over 0.5%. These levels depend on the incubation and storage conditions. Metabolites should be formed in certain proportions to obtain a good flavor. Some conversions are detrimental to the quality; an example is the formation of acetic acid from alcohol by the yeasts after uptake of oxygen from the air.

In the traditional manufacture of kefir, milk with added active grains is first kept for some time at a temperature of 20–25°C to enhance the lactic fermentation. Subsequently, the grains are sieved out of the milk and the milk is further ripened at a temperature of 8–10°C, which stimulates the alcoholic fermentation. Modern ways of processing use homogenized, pasteurized whole or standardized milk. The milk is not inoculated with the grains as such but with soured milk obtained by sieving a previously fermented culture of grains. A certain amount of L starter may also be added. The inoculated milk is put in well-closed packages and incubated. In this way, ''firm kefir'' is obtained. A considerable amount of gas forms during the fermentation. Incubation time and temperature determine the properties of the final product, i.e., amounts of lactic acid, alcohol and CO_2, and aroma. In the manufacture of ''stirred kefir,'' the milk is fermented at a fairly high temperature, slowly cooled while stirred, further ripened at low temperature, and packed. Modern packing materials, e.g., aluminum foil–capped plastic cups, cannot resist a high CO_2 pressure and ballooning can readily occur. Accordingly, a hole is made in the foil, or the fermentation is stopped at an earlier stage, at the expense of the traditional characteristics. Continuous production of kefir is also possible. A substitute for kefir can be obtained by adding sucrose to buttermilk (e.g., 20 g/L) together with the yeast *Saccharomyces cerevisiae* and incubating it for 3–4 days at 18–21°C in a closed firm package.

KUMISS is a well-known milk drink in Russia and western Asia. The cultured milk formerly was valued because of its supposed control of tuberculosis and typhus. The product is traditionally made of mares' milk. The fermenting flora is variable, as in kefir.

Kumiss is a sparkling drink. It contains 0.7% to 1% lactic acid, 0.7% to 2.5% alcohol, 1.8% fat, and 2% protein; it has a grayish color. During its manufacture protein is substantially degraded. Together with the fermentation com-

pounds formed, the proteolysis is responsible for a specific flavor. The fermentative processes must proceed in such a way that the metabolites are formed in certain proportions.

Traditional kumiss is not manufactured on an industrial scale. To raw mares' milk, temperature 26–28°C, 40% starter is added, which increases the acidity to 50°N. (The starter is propagated as a kind of continuous culture in mares' milk.) The mixture is intensely stirred and subsequently left undisturbed, which raises the acidity to 60°N. The milk is stirred for an additional hour, to aerate it and to obtain dispersed protein particles, and it is bottled. The bottles are kept for a few hours at 18–20°C and then for a certain time at 4–6°C, which temperature sequence enhances the lactic and alcoholic fermentations. An imitation product of kumiss is now being made on an industrial scale, starting from cows' milk. Compared to mares' milk, cows' milk has a high ratio of casein to serum proteins and a low lactose content (see Table 1.5). The composition of mares' milk is therefore simulated by mixing cows' milk and ultrafiltered, heated whey; heating of the whey is necessary to inactivate rennet. The starter contains *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Candida kefir*.

20.3 YOGURT

Yogurt is probably the most popular fermented milk. It is made in a variety of compositions (fat and dry matter content), either plain or with added substances: fruits, sugar, gelling agents. Beverages and edible ices derived from yogurt are also produced. The manufacture of yogurt will be discussed here to exemplify the problems met in the making of fermented milks.

20.3.1 The Yogurt Bacteria

20.3.1.1 Growth

The essential flora of yogurt consists of the thermophilic lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. For a satisfactory flavor to develop, approximately equal numbers of both species should be present (see below). They have a stimulating effect on each other's growth (protocooperation). The proteolytic rods enhance growth of the streptococci by forming small peptides and amino acids, the main amino acid being valine. Milk contains too little of these amino acids and the cocci, which are very weakly proteolytic, form the acids too slowly. The cocci enhance the growth of the rods by forming formic acid out of pyruvic acid under anaerobic conditions, and by a rapid production of CO_2 (see Section 11.1). The stimulatory effect of formic acid remains unnoticed in intensely heated milk because in this milk formic acid has been formed by decomposition of lactose. The production of formic acid by the cocci is, however, essential in industrial practice, where more moder-

ate heat treatments of yogurt milk are applied, e.g., 5–10 min at 85°C. Due to mutual stimulation during combined growth of the yogurt bacteria in milk, lactic acid is produced much faster than would be expected on the basis of the acid production by the individual pure cultures. Some antibiosis also occurs in yogurt in that the cocci cannot grow after a certain acidity has been reached. The rods are less susceptible to acid and continue to grow. Protocooperation and antibiosis are of great importance in the growth of the yogurt bacteria as well as for the quality of yogurt (see also Fig. 20.1).

The cocci as well as the rods contribute significantly to the properties of yogurt. The properties of the bacterial strains used should be matched to each other because not every combination of strains is suitable. Furthermore, both species should be present in large numbers in the product, hence in the starter. The optimum ratio of diplococci to rods depends on the properties of the strains and is often approximately 1:1. This ratio between the yogurt bacteria is best maintained if the inoculum percentage is, say, 2.5, the incubation time 2.5 h at 45°C, and the final acidity some 90–100°N. The growth of cocci and rods in yogurt, incubated under these conditions, is depicted in Figure 20.2. The ratio between the species keeps changing. Initially, the streptococci grow faster due to the formation of growth factors by the rods, and probably also due to the latter compounds being added via the inoculum (especially in the manufacture of set
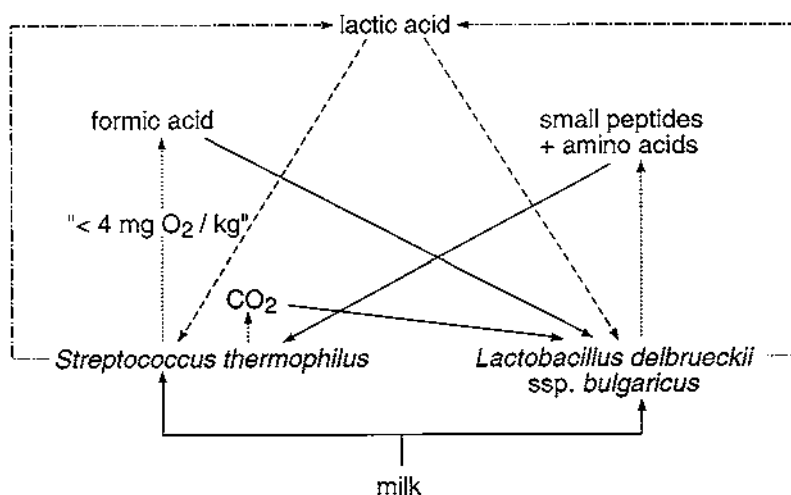
FIGURE 20.1 Outline of the stimulation and the inhibition of the growth of yogurt bacteria in milk. - · - · -, formation of lactic acid; · · · · · , formation of growth factors; ————, stimulation; — — —, inhibition. After F. M. Driessen, International Dairy Federation, Bulletin No. 179, 107–115 (1984).

log *N*
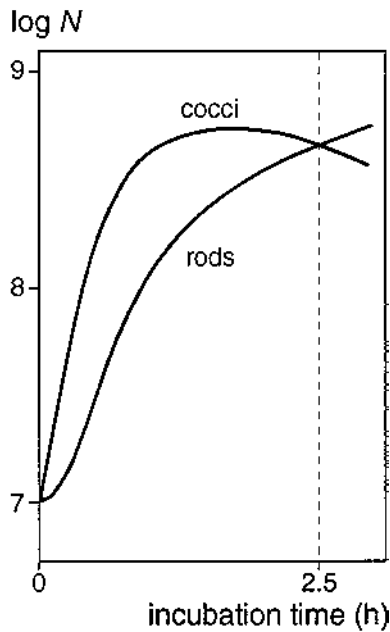
FIGURE 20.2 Growth of cocci and rods in yogurt (starter) cultured at 45°C in intensely heated milk. Inoculation percentage equals 2.5. *N* = count in ml^{-1}. Approximate results.

yogurt). Afterward, the cocci are slowed down by the acid produced. Meanwhile, the rods have started to grow faster because of the growth factors (CO_2 and formic acid) formed by the cocci. As a result, the original ratio is regained. The yogurt should then have attained the desired acidity. Continued incubation or inadequate cooling causes the rods to become preponderant.

Varying the above-mentioned conditions during incubation changes the ratio between the rods and the cocci as follows:

a. *Incubation time*. A shorter incubation time, which means a lower acidity, will cause the yogurt to contain too high a proportion of cocci. Transferring a yogurt starter repeatedly after short incubation times may cause the rods to disappear from the culture. Conversely, long incubation times will cause an increasing preponderance of the rods.

b. *Inoculum percentage*. Increasing the inoculum percentage will enhance the rate of acid production. The acidity at which the cocci are slowed down will thereby be reached earlier, resulting in an increased number

of rods (incubation time being the same). At a lower inoculum percentage, the ratio between the bacteria will shift in favor of the cocci.

c. *Incubation temperature*. The rods have a higher optimum temperature than the cocci. Incubation at a little higher temperature than 45°C will shift the ratio in favor of the rods; incubation at a lower temperature will enhance the cocci.

Obviously, a correct ratio between the species in the starter can be maintained, or be recovered if need be, by proper selection of the propagation conditions. Currently, concentrated starters are increasingly used, ensuring a correct bacterial composition of the starter.

20.3.1.2 Metabolites

S. thermophilus and *L. delbrueckii* ssp. *bulgaricus* form products that contribute to the flavor of yogurt as well as to its structure and consistency. The following are the main compounds involved:

a. *Lactic acid*. Both bacteria form lactic acid out of glucose. Galactose, formed during the decomposition of lactose, is not converted. Hence, the molar concentration of galactose increases just as much as the lactose content decreases. See item d.

By far the greatest part of glucose is decomposed in a homofermentative way. *S. thermophilus* forms L(+) and *L. delbrueckii* ssp. *bulgaricus* D(−) lactic acid. The isomers are produced in almost equal quantities. (Section 20.1.2 mentions physiological aspects with respect to the consumption of lactic acid.) CO_2, acetic acid, and ethanol are also produced, be it in small amounts. The acetic acid content of yogurt is $0.03–0.05$ kg $\cdot$ m^{-3} ($0.5–0.8$ mM) and the ethanol content $0.01–0.04$ kg $\cdot$ m^{-3} ($0.2–0.7$ mM). Ethanol has a relatively high flavor threshold and it likely does not contribute to the flavor of yogurt. The lactic acid content of yogurt is $0.7–0.9\%$ w/w ($80–100$ mM).

b. *Acetaldehyde* (ethanal). This component is essential for the characteristic yogurt aroma. Most of it is formed by the rods. An important precursor is threonine (see Section 11.1), which is a natural component of milk, be it at low concentration. In addition, proteolysis by the lactobacilli yields threonine. The content of acetaldehyde of yogurt is about 10 mg $\cdot$ kg^{-1} (0.2 mM).

c. *Diacetyl* ($CH_3 \cdot CO \cdot CO \cdot CH_3$). *S. thermophilus* and, to a lesser degree, *L. delbrueckii* ssp. *bulgaricus* form diacetyl in a way that probably corresponds to the mechanism followed by leuconostocs and by *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* (Section 11.1). The yogurt bacteria do not decompose citric acid. Hence, pyruvic acid, formed

during sugar fermentation, is the only precursor of diacetyl. The diacetyl content of yogurt ranges from 0.8 to 1.5 mg · kg^{-1} (0.01–0.02 mM).

d. *Polysaccharides*. The yogurt bacteria can form a ''hairy'' layer or glycocalix, which predominantly consists of polysaccharide chains, made up of galactose and other glucides. They can be partially secreted into the liquid and are then called *exopolysaccharides*. The polysaccharides play an important role in yogurt consistency, especially of stirred yogurt (see below). Although various strains show quite a variation in the amount of polysaccharide produced, this variation does not correlate well with the consistency obtained. Presumably, the type of polysaccharide produced is of greater importance.

20.3.2 Manufacture; Set and Stirred Yogurt

Traditionally, *set yogurt* was made of concentrated milk. The milk was heated on an open fire until, say, one-third of the water had evaporated. Then the milk was allowed to cool, and when a temperature of about 50°C was reached, the milk was inoculated with a little yogurt. After fermentation, a fairly firm gel is obtained. A similar process is still being used, but either the milk is evaporated under vacuum or some milk powder is added (see Fig. 20.3). One may use the same process for making set yogurt from nonconcentrated milk. The yogurt obtained is less rich in flavor, is far less firm, and is prone to syneresis (wheying off). Generally some gelling agent is added to prevent syneresis and to enhance firmness, especially if pieces of fruit are added. Another difference between both products is the titratable acidity. Since a satisfactory flavor and texture are only obtained at a pH below, say, 4.5 and the concentrated milk has a greater buffering capacity, the latter is fermented to an acidity of about 130°N, versus 90–100 °N for nonconcentrated milk (see Section 11.2).

Another type is *stirred yogurt*, virtually always made from nonconcentrated milk. After a gel is formed, it is gently stirred to obtain a smooth and fairly thick, but still pourable, product (see Fig. 20.3). There are other differences in the manufacturing process. Set yogurt is fermented after being packed, implying that final cooling has to be achieved in the package. Stirred yogurt is almost fully fermented before it is packed. Another difference is that only certain strains of yogurt bacteria produce the correct consistency or thickness after stirring, and only so when incubating at a fairly low temperature. However, the bacteria make less of the desired flavor compounds at lower temperatures. In order to ensure that stirred yogurt has a distinct yogurt flavor, it is necessary that the starter be propagated under the same conditions as for set yogurt. This means at about 45°C, and with such an inoculum size and incubation time as to reach about equal numbers of cocci and lactobacilli.

The rate of acidification greatly differs between set and stirred yogurt, due
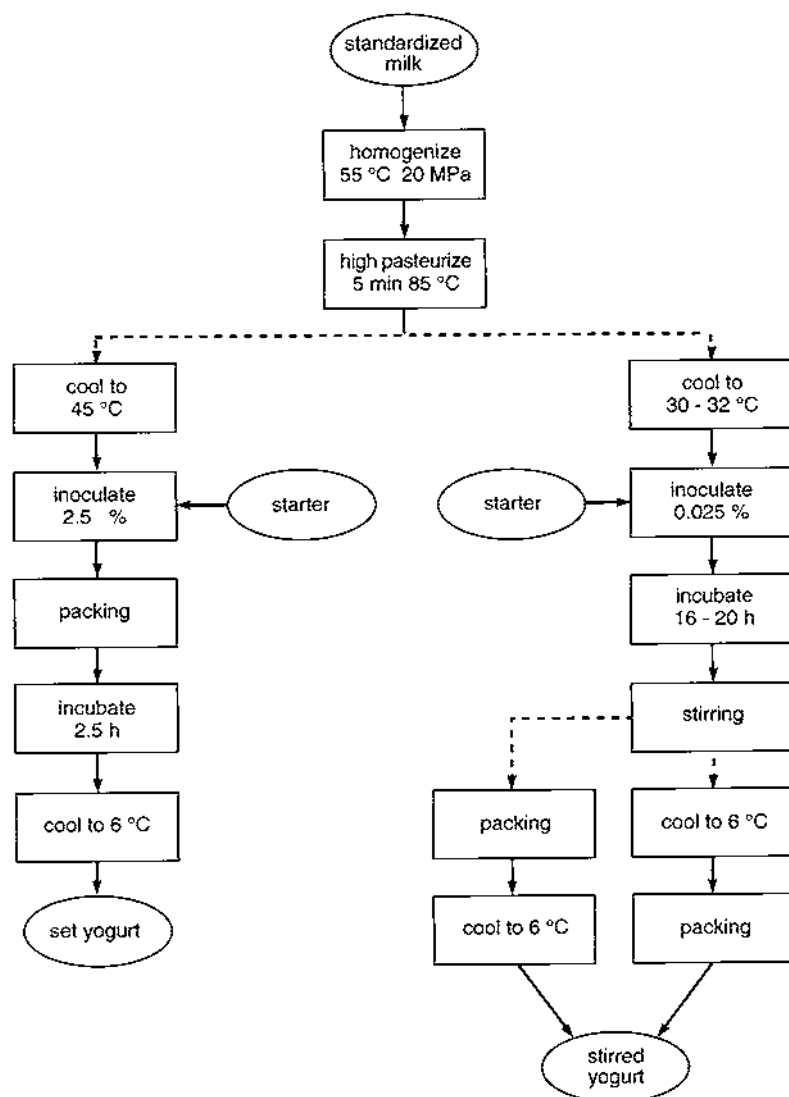
FIGURE 20.3 Examples of the manufacture of set yogurt and of stirred yogurt. Set yogurt is often made from concentrated milk ($Q \approx 1.4$).

to the differences in inoculum size and incubation temperature. Examples are given in Figure 20.4, curves 1 and 3. Curve 2 gives the gelation as a function of time, hence of pH, for set yogurt. It is seen to start when the pH reaches about 4.7 and that the stiffness then rapidly increases, to reach a fairly high value in about 20 min. When making stirred yogurt, gelation begins at about the same pH but it takes a longer time before the gel has become sufficiently firm for the stirring to be started.

　　　Acidification will go on, albeit slowly, after the product has been cooled (Section 11.2). To minimize or even prevent ongoing acidification, stirred yogurts or yogurt-like products are sometimes pasteurized; this also prevents growth of any yeasts and molds present. To allow pasteurization without the product becoming inhomogeneous, it is necessary to add thickening agents (pectins, modified starch, gelatin).

　　　Processes for continuous, i.e., flow-through, manufacture of stirred yogurt have been developed. This would have considerable advantages, such as better control of fermentation and further manufacture, smaller product losses, and more efficient use of water and energy. For the manufacture of set yogurt, continuous
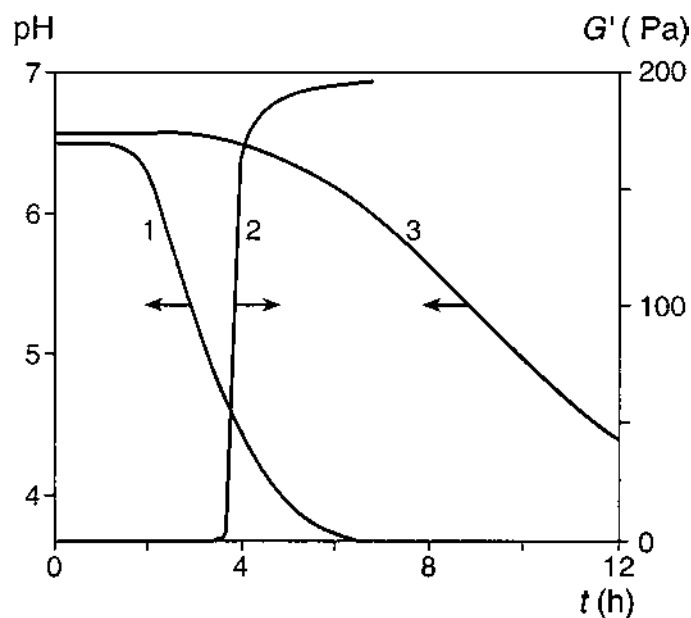
Figure 20.4　Relation between pH or elastic modulus (G') and incubation time (t) during yogurt manufacture. Curves 1 and 2, set yogurt; curve 3, stirred yogurt. Approximate examples.

preincubation, to a stage where no setting occurs as yet, is also possible; to prevent texture defects, the pH at the stage of packing should not be lower than about 5.2.

20.3.3 Physical Properties

As mentioned, the physical structure of yogurt is a network of aggregated casein particles (see also Section 3.2.3) onto which part of the serum proteins have been deposited due to their heat denaturation. The network encloses fat globules and serum. The largest pores of the network are of the order of 10 μm. The existence of a continuous network implies that yogurt is a gel, a viscoelastic material characterized by a fairly small yield stress (say, 100 Pa). If the gel is broken up, as in the making of stirred yogurt, a fairly viscous non-Newtonian liquid can be formed; it is strongly shear rate thinning and thus has an apparent viscosity. Set and stirred yogurt have markedly different textures (see also Section 23.6 for a discussion of rheological properties).

20.3.3.1 Firmness of Set Yogurt

Firmness of set yogurt is often estimated by lowering a probe of given weight and dimensions into the product during a given time. The reciprocal of the penetration depth then is a measure of firmness. Firmness is not closely related to an elastic modulus but rather to a yield stress. Its value depends on the method of measurement, especially the time scale, and on several product and process variables (see also Fig. 20.5):

   a. *Casein content of the milk*. Firmness is about proportional to casein content cubed. Natural variation in casein content can thus have a
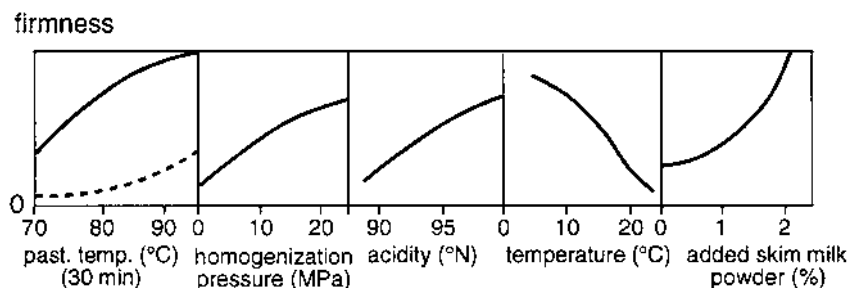
FIGURE 20.5 The influence of some product and process variables on the firmness (reciprocal of the penetration depth of a ball) of set yogurt. The broken line refers to yogurt made of nonhomogenized milk. Approximate examples.

marked effect. Evaporating the milk, adding skim milk powder, or partial ultrafiltration increase firmness.

b.   *Fat content*. The higher the fat content, the weaker the gel because the fat globules interrupt the network.

c.   *Homogenizing* the milk, however, leads to a much enhanced firmness because the fat globules then contain fragments of casein micelles in their surface coat, by which they can participate in the network upon acidification (see also Section 8.6). The volume fraction of casein is thus effectively increased. (Homogenization of skim milk makes no difference.)

d.   *Heat treatment* of the milk considerably enhances firmness. The deposition of denatured serum proteins increases the volume fraction of aggregating protein; it also may alter the number and the nature of the bonds between protein particles. Milk is generally heated for 5–10 min at 85–90°C.

e.   *Yogurt cultures* vary in the firmness they produce (at a given acidity), but as a rule the differences are small.

f.   *The pH*. Generally, the yogurt is firmer at a lower pH. The preferred pH is between 4.1 and 4.6.

g.   *Incubation temperature*. The lower it is, the longer it takes before a certain pH, and thereby a certain firmness, is reached, but the finished product is much firmer.

h.   *Temperature of the yogurt*. For the same incubation temperature, a lower measuring temperature gives a greater firmness. The effect is quite strong (see Fig. 20.5). The explanation presumably is that the casein micelles swell when the temperature is lowered (and vice versa); since the particles are essentially fixed in the network and since the network cannot swell, this would imply that the contact or junction area between any two micelles is enlarged, by which a greater number of bonds are formed per junction.

20.3.3.2   Syneresis

Syneresis of casein gels is discussed for rennet-induced gels in Section 21.3.6. Briefly recalling that, syneresis is for the most part due to a rearrangement of the network, leading to an increase in the number of particle–particle junctions. The network then tends to shrink, thereby expelling interstitial liquid. Acid casein gels are not very prone to syneresis, especially not at pH values in the range 4–5. In yogurt, syneresis is, of course, undesirable.

The tendency to exhibit syneresis greatly depends on the incubation temperature. If milk is incubated at 20°C (with a mesophilic starter, since yogurt bacteria hardly grow at that temperature) so that the gel is formed at that temperature, absolutely no syneresis occurs, whereas when incubating at 32°C it is possible.

When incubating at 45°C syneresis can only be prevented if the milk has been intensively heated, especially if its casein content has also been increased and the storage temperature is low. However, if the package containing the product is even slightly shaken at a time when gel formation has just started and the gel is still weak, it may fracture locally with copious syneresis occurring subsequently. If the top surface of the set yogurt is wetted, e.g., because water is condensed on the inside of the lid of the package and a few drops fall off, some whey separation may be induced. If the pH of the yogurt has fallen below 4 some syneresis may also occur, especially if the temperature is fairly high and the package is shaken. Containers made of a material to which the gel formed does not stick will readily induce whey separation between the wall and the product.

In the production of stirred yogurt, significant syneresis will lead to a poor product. The stirring breaks the gel into lumps and these then would immediately start syneresing. An inhomogeneous mixture of lumps in whey is formed; further stirring would break down the lumps and make a smoother product, but it would then become insufficiently viscous. To prevent this, it is necessary to incubate the milk at a low temperature, e.g., 32°C or even lower if the casein content of the milk is low.

20.3.3.3   Viscosity of Stirred Yogurt

Stirred yogurt should be smooth and fairly viscous. A good product also gives the impression of being ''long'' or ''stringy'': When slowly pouring it, a fairly thin thread readily forms that behaves somewhat elastically when it breaks. Viscosity is easiest determined by means of a Ford cup; a given amount of yogurt is allowed to flow from an opening at the conical lower end of a cup, and the time needed for that is a measure for the viscosity.

The product is strongly shear rate thinning, as illustrated in Figure 20.6. The figure also shows considerable hysteresis. After a high shear rate is applied, the apparent viscosity at lower shear rates is permanently decreased and the viscous behavior becomes closer to Newtonian. This implies a lasting structural breakdown. (For completeness, the viscosity increases a little on prolonged standing.) This is all in agreement with the behavior of a liquid containing gel fragments. The viscosity increases with the viscosity of the continuous liquid (''solvent'' or ''whey'') and with the volume fraction ϕ of gel fragments. The latter is larger than the volume fraction of casein particles because the fragments contain a lot of interstitial solvent. More intensive stirring (a higher shear rate) further breaks down the gel fragments and also gives them a more rounded shape, thereby decreasing effective ϕ.

The apparent viscosity at a given shear rate of stirred yogurt depends on:

a. *Firmness of the gel before stirring*. The higher it is, the larger ϕ after stirring. The factors determining firmness have been listed and discussed above.
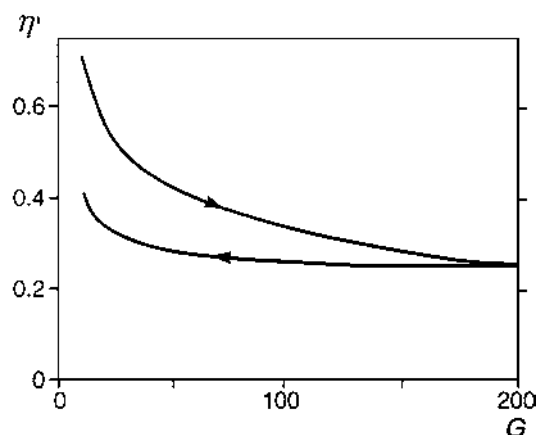
FIGURE 20.6 Example of the apparent viscosity (η' in Pa · s) of stirred yogurt as a function of the shear rate (G in s^{-1}), before and after shearing at 200 s^{-1}.

b. *Intensity of stirring*. The more vigorous the stirring, the lower the apparent viscosity but also the smoother the product. Consequently, a high gel firmness is needed to allow fairly vigorous stirring without the product becoming too thin.

c. *Syneresis*. The more syneresis occurs after stirring, the less viscous and more lumpy the product becomes. The tendency to show syneresis is less for a firmer gel and especially for a lower incubation temperature.

d. *Bacterial strains applied*. It is tempting to assume that a greater production of (exo)polysaccharides results in a higher viscosity of the solvent, hence of the yogurt. The increase in solvent viscosity is, however, very small and the increase in product viscosity does not correlate with polysaccharide production. Nevertheless, considerable variation occurs among strains. It appears that this is mainly due to a variation in inhomogeneity of the gel formed. An inhomogeneous gel readily gives large lumps on stirring, and the more homogeneous it is, the more viscous and smooth the stirred yogurt. How the bacterial polysaccharides affect the gel inhomogeneity is currently not quite clear.

Vigorous agitation of stirred yogurt during further processing must be avoided to prevent the product from becoming too thin. Packing machines can be especially damaging.

20.3.4 Flavor Defects and Shelf Life

A main quality problem with yogurt is that souring tends to go on after delivery to the retailer, and the product may be too acidic when consumed. Moreover, the yogurt may become bitter due to excessive proteolysis; this would also depend on the starter strains used. The development of these defects generally determines the shelf life. Of course, the product is cooled to slow down acidification, but it is difficult to cool fast enough. Set yogurt is present in a package and cannot be stirred; stirred yogurt should not be stirred too vigorously because it would then become too thin. And even at refrigerator temperatures, acidification and other changes caused by the enzyme systems go on, albeit slowly.

Other defects may be caused by contaminating organisms, mainly yeasts and molds. The off-flavors may be characterized as yeasty, fruity, musty, cheesy, or bitter, and, occasionally, soapy-rancid. A flavor threshold is generally reached at a count of about 10^4 yeasts + molds per ml. The growth of these microbes is largely determined by the amount of oxygen available, hence by the head space volume and the air permeability of the container.

Another defect is too little of the characteristic flavor (which is of less importance in yogurts with added fruits). It may be due to a low incubation temperature, an excessive growth of the streptococci, or by the lactobacilli being weak aroma producers. Insufficient acidification, e.g., because the milk is contaminated with penicillin, also leads to a bland product.

Finally, off-flavors in the milk used for manufacture may naturally cause flavor defects in the product.

SUGGESTED LITERATURE

- Several aspects of manufacture and properties of fermented milks are discussed in:
  Fermented milks: Science and Technology, Bulletin of the International Dairy Federation No. 227, 1988.
- Several fermented milk products are discussed at an elementary level in:
  F. V. Kosikowski and V. V. Mistry, *Cheese and Fermented Milk Foods*, 3rd ed., two volumes, published by the authors, 1997.
- A general treatment of yogurt and its manufacture is given by:
  A. Y. Tamime and R. K. Robinson, *Yoghurt: Science and Technology*, Pergamon Press, Oxford, 1985.
- Much about industrial yogurt manufacture can be found in:
  R. K. Robinson, ed., *Modern Dairy Technology*, Vol. 2, *Advances in Milk Products*, 2nd Ed., Elsevier, London, 1993.

IV

CHEESE

21

Principles of Cheese Making

When fresh milk is left to become sour, the casein aggregates. If souring occurs at not too low a temperature and without stirring or shaking of the milk, a gel is formed. When keeping the gelled or clotted milk, some whey separation (syneresis) generally occurs. Syneresis can be enhanced by heating and stirring; the mass then separates into curd and whey. By allowing more of the whey to leak from the curd, e.g., by hanging the curd in a cloth, a primitive fresh cheese is obtained (baker's cheese, quarg, or simply ''curds'').

This may be the origin of cheese making. However, milk has also been clotted for centuries by the addition of specific agents, especially rennet, an extract of calf stomach. This may have been discovered when milk was stored in stomachs. Various vegetable clotting agents exist also; milk can be clotted by stirring it with twigs from a fig tree. All of these types of clotted milk show syneresis. Overall, the art of transforming milk into curd and whey is very old. It will always have been accompanied by acidification, caused by lactic acid bacteria.

To make a real cheese, other process steps are needed: shaping (often by pressing), salting, and curing. These steps could readily evolve, and the evolution has led to a great variety of cheese types. However, they have a few things in common:

    a. The greater part of the casein and the fat of the milk are concentrated in the cheese, which is thus a very nutritious product.
    b. Cheese keeps much longer than milk, and also longer than fermented milks. During keeping (curing) it changes in properties; this is called *ripening* or *maturation*.

c. Cheese generally has a distinct and characteristic flavor, due to a great number of flavor compounds formed during ripening. Ripening in particular shows great variation.

21.1 INTRODUCTION

When milk is made into cheese, casein and fat are concentrated, whereas the other milk components, especially water, are mainly removed with the whey. None of the milk components is fully retained and other substances may be added, notably salt. This is illustrated in Figure 21.1. The yield and the composition of the cheese are determined by the properties of the milk, especially composition, and by the manufacturing practice.

Cheese manufacture is aimed at making an attractive and durable product, in which important nutrients of the milk are concentrated. The cheese must be left to ripen to acquire desirable flavor and consistency. To achieve this, the cheese is kept for a variable time under favorable conditions. The storage conditions vary widely with the type of cheese involved, but temperature and humidity are usually such that most foods would spoil. Composition and properties of the cheese must be such that deterioration is prevented. All operations and treatments must therefore be such that a product is obtained that can be matured satisfactorily and does not spoil.

Cheese is more durable than milk, and often also more durable than sour milk. Its shelf life varies from a few days to several decades. A combination of factors is responsible for the keeping quality: absence of sugar, low pH, lactic acid, salt, anaerobic conditions, and, often, a protective rind.

Cheese making is a complicated process, involving many process steps and several biochemical transformations. All of these variables affect yield, composition, and quality of the cheese and its byproducts (predominantly whey), and often in different directions. Consequently, optimization of cheese making is intricate. Even the control of the composition of the end product, a rather straightforward activity for most dairy products, is far from easy to achieve in cheese making. Moreover, the way of processing may strongly affect production costs: labor, equipment, product loss, etc.

Overall, a full understanding of the various physical and chemical transformations, their interdependence, and the ways in which they can be affected is needed if one wants to base cheese making on scientific principles rather than on empirical rules of thumb. Because several widely differing disciplines are involved—process engineering, physical chemistry, biochemistry, and microbiology, to name a few—our understanding is far from complete. Nevertheless, the remainder of this book is devoted to providing the reader with insight into the essential principles.
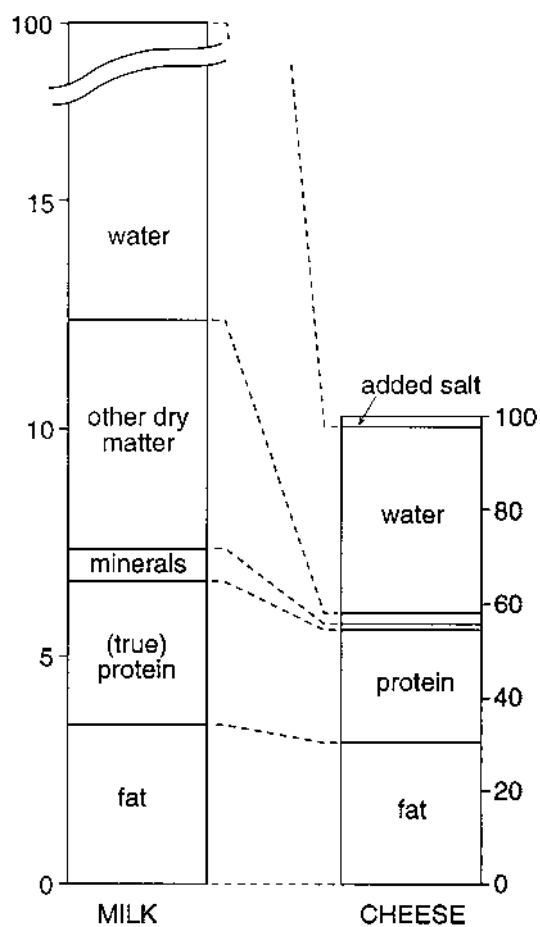
FIGURE 21.1 Example of the gross composition of milk and cheese and of the transfer of components from milk to cheese.

21.2 ESSENTIAL PROCESS STEPS

The manufacture of cheese may involve many different process steps, where some steps are essential for all cheese varieties:

a. *Renneting of the milk* by means of enzymes or acid, or both. A gel is formed, due to the casein particles aggregating into a network, enclosing fat globules.

b. *Removal of the whey* (comparable to milk serum), by means of synere-

  sis of the gel. The resulting curd makes up 10% to 30% of the original volume of milk. The drier the curd, the firmer and the more durable the cheese will be.

c. *Acid production* in the cheese during its manufacture, due to the conversion of lactose into lactic acid by lactic acid bacteria. The resulting pH of curd and cheese affects such parameters as syneresis, consistency, and ripening (flavor development) of the cheese.

d. *Salting*. Cheese contains added NaCl, mostly 1% to 4%. That does not apply to some fresh-type cheeses such as quarg. The salt affects durability, flavor, and consistency of the cheese.

e. *Fusion of curd grains into a coherent loaf* that is easy to handle. Often the cheese has a rind, which may protect the interior. Pressing enhances the formation of a closed rind.

f. *Ripening*. Microbial, biochemical, chemical, and physical processes during ripening are responsible for changes in composition and structure of the cheese; hence flavor and texture.

The last two process steps are typical of ripened cheese; when these are not carried out, the product is referred to as fresh cheese.

  Nowadays some additional process steps are commonly applied, their main objective being to diminish variation in the course of the manufacture of the cheese and in its properties. This concerns:

  Pasteurization of the cheese milk. Pasteurization destroys microorganisms and enzymes that are harmful for the ripening. It may also serve to kill pathogens because some of these bacteria can survive for some time, especially in soft-type cheeses.

  Addition of cultures of microorganisms to the cheese milk, especially starters of lactic acid bacteria. The addition is essential if the cheese milk has been pasteurized but is also highly desirable for cheese made of raw milk. The composition of the starter depends on the type of cheese to be made. For some varieties, other specific microorganisms are also added.

The main aim of modern cheese making is to process the milk rapidly, often maintaining a rigid time schedule, and to precisely adjust the composition of the cheese to control yield and quality. This has greatly altered the cheese-making operation, but generally without fundamentally changing the processes involved.

21.3 CLOTTING AND SYNERESIS

When milk turns sour or when a suitable proteolytic enzyme is added, or when both treatments occur, the milk clots, i.e., the casein particles aggregate and form a gel. This occurs during the manufacture of fermented milks and cheese, as well

as in milk in the stomach. Clotting thus is of great importance. Here we will especially deal with the rennet coagulation, i.e., clotting by enzymes isolated from the abomasum of calves. The most important enzyme in rennet is chymosin.

Chymosin brings about a splitting of κ-casein in such a way that the ''hairs'' protruding from the casein micelles disappear or, more precisely, become a lot shorter. The released caseinomacropeptide (formerly called whey proteose) dissolves, whereas para-κ-casein remains in the micelles. The altered casein is referred to as paracasein; it cannot be dissolved or be dispersed in milk serum. Because of this, the paracasein micelles in the milk coagulate, provided that the Ca^{2+} activity suffices. Several other proteolytic enzymes can bring about the same reaction, but they often cause problems in ripening of the cheese.

21.3.1 Chymosin

Chymosin (EC 3.4.23.4, MW ≈ 35600, isoelectric pH ≈ 4.65) is a soluble protein. It is an aspartate proteinase, thus an endopeptidase, which means that it can split proteins into relatively large fragments. Chymosin is related to the common stomach enzyme pepsin (EC 3.4.23.1), which commercial calf rennets always contains (say, 20% of the proteolytic activity). Unlike pepsin, chymosin cannot hydrolyze the immunoglobulins of colostrum (which explains why the newborn calf produces chymosin and no pepsin). Incidentally, in the calf stomach inactive prochymosin is synthesized, which converts itself to the active form by means of directed proteolysis.

A lower pH enables chymosin to hydrolyze more peptide bonds, but at pH 6.7 especially the Phe-Met bond between residues 105 and 106 of κ-casein is split. This is a fast reaction. Presumably, the positive charge of that region of the peptide chain (see Fig. 2.18), as well as the easy accessibility of that region, accounts for the strong affinity toward the negatively charged active site of the enzyme. Synthetic peptides comprising some amino acid residues around the Phe-Met bond of κ-casein are also readily hydrolyzed by chymosin. The latter hydrolysis can be applied in tests for estimating the strength of rennet preparations.

Some chymosin becomes adsorbed onto paracasein, whereby part of it is included in the cheese, the amount increasing with decreasing pH. At pH 6.7 the adsorption is very weak.

Chymosin is readily inactivated under certain conditions (see Fig. 21.2). At low pH this must be attributed to autocatalysis (the enzyme decomposes itself), at high pH to denaturation. In fresh milk significant inactivation occurs at 45°C. Salt inhibits the inactivation, hence the high salt content of commercial rennet.

21.3.2 The Enzyme-Catalyzed Reaction

The rate of the enzyme reaction cannot be described on the basis of the Michaelis-Menten equation; the reaction is first order, both with respect to concentration
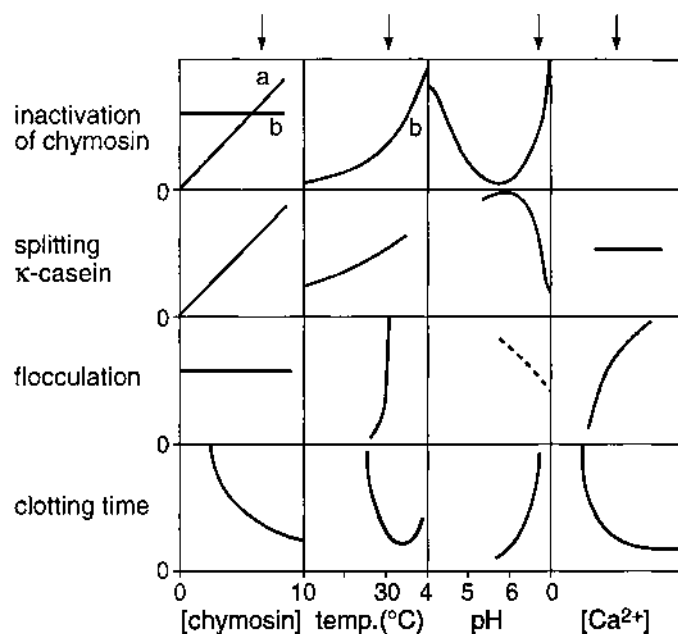
FIGURE 21.2 Effect of chymosin concentration, temperature, pH, and Ca²⁺ concentration on the rates of chymosin inactivation, splitting of κ-casein (in milk), flocculation of paracasein micelles (this implies that κ-casein has been completely split), and the clotting time of milk. Meant to illustrate trends. (a) at pH 3.5; (b) at pH 7; a blank space implies no or little effect, a broken line a rough estimate; arrows indicate the conditions as often used in making cheese from fresh milk.

and to time, at least at physiological pH. This must be ascribed to the great difference in size, hence in diffusion coefficient, between enzyme molecules and casein micelles. The latter, as it were, do not move and the enzyme must approach them by means of diffusion. During commercial renneting, one enzyme molecule is available for, say, 30 micelles. The reaction velocity thus is ''diffusion-limited.'' There is a random removal of the hairs. The course of the reaction is depicted in Figure 21.3, curve S.

Some factors affecting the reaction velocity are illustrated in Figure 21.2. The effect of temperature is relatively slight and corresponds to the dependence of the diffusion coefficient (through the viscosity) on temperature. The calcium ion activity has little effect. A certain ionic strength is needed, and that of milk is appropriate. The pH has a considerable effect. At decreasing pH the affinity
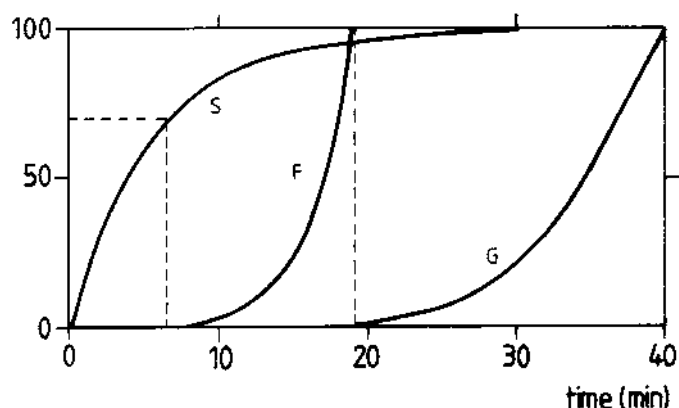
FIGURE 21.3 Renneting of milk. Percentage of κ-casein split (S), degree of flocculation of paracasein micelles (F, e.g., deduced from the viscosity increase), and shear modulus of the formed gel (G), as a function of the time after adding rennet. F and G, arbitrary scale. Only meant to illustrate trends. At physiological pH and about 30°C.

of the enzyme for the micelles increases and leads to an increased reaction velocity. At a still lower pH the velocity is, however, smaller, presumably because the enzyme is now adsorbed so strongly that it takes considerable time before an adsorbed molecule is released again and can diffuse further (this implies that the reaction is no longer diffusion-limited). An additional effect of the increased adsorption at low pH is that the hairs are not truly removed at random, but the enzyme tends to form ''bare patches'' on a micelle before desorbing and diffusing away.

21.3.3 The Flocculation

Basic aspects are discussed in Section 3.2.3. The micelles only flocculate when by far the greater part of the hairs has been removed, so that the steric repulsion of the micelles has been sufficiently diminished. This is illustrated in Figure 21.3, where flocculation occurs when about 70% of the κ-casein has been split. The more hairs that have been removed, the faster the flocculation proceeds. This is illustrated in Figure 21.4, in which the estimated relative flocculation rate (which essentially is a measure for the reciprocal of the stability factor W; see Section 3.2.3) is given as a function of the degree of splitting.

The flocculation, i.e., the sticking together of paracasein micelles, is partly due to van der Waals attraction, but this attraction is in itself insufficient. The required Ca^{2+} activity already points to this. Furthermore, the effect of the Ca^{2+}

relative flocculation rate
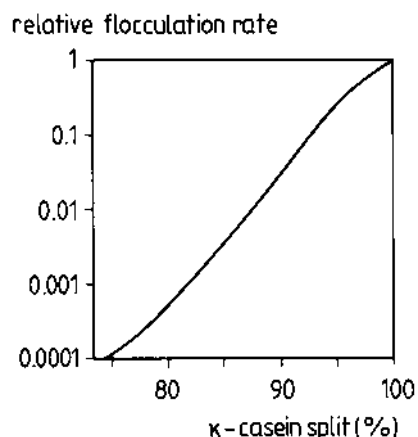
FIGURE 21.4   Approximate flocculation rate of micelles during clotting at 30°C as a function of the percentage of the κ-casein that has been split. ''Relative'' implies: as compared to true paracasein micelles at the given conditions (pH, temperature, Ca^{2+} activity, etc.).

ions is twofold. First of all, they diminish the electrostatic repulsion by neutralizing negative charges on the micelles. In the pH range concerned, Ca^{2+} ions act more effectively than H^+ ions (incidentally, lowering the pH of milk considerably increases its Ca^{2+} activity; see Fig. 2.9). Second, the Ca^{2+} can make bridges (salt linkages) between negative sites on the paracasein micelles, in addition to the bridges between positive and negative sites that can be formed.

Figure 21.2 illustrates some factors affecting the flocculation rate of paracasein micelles (κ-casein completely split). In addition to the Ca^{2+} ions, temperature also has a considerable effect. At 20°C flocculation does not occur at all. At 60°C the rate is almost as fast as determined by the encounter frequency; in other words, the stability factor *W* hardly surpasses unity (at least if the Ca^{2+} activity is sufficiently high). The mentioned stabilization at lower temperature presumably is to be ascribed to protruding hairs of β-casein; such protrusion depends closely on temperature (see Section 3.2.3).

21.3.4   The Renneting Time

When rennet is added to milk it takes a while before the micelles start to flocculate (Fig. 21.3), but from that time on the flocculation rate increases rapidly (Fig. 21.4). At a certain moment, flocs can be detected by eye. The time required may be defined as the renneting time. Alternatively, the renneting time can be taken as the time needed to form a gel, or to form a gel of a given firmness (see Fig.

22.4). Figure 21.3 shows that the modulus, and thereby the firmness, increases rapidly. However the renneting time is defined, it is an important parameter in cheese making. Figure 21.2 illustrates its dependence on some factors.

The renneting time is almost inversely proportional to the enzyme concentration. The relationship, known as the rule of Storch and Segelcke, does not fit precisely (this depends in part on the definition of the renneting time), nor can it be explained in a simple manner. The complicated combined action of the enzyme reaction and the flocculation (which increases in rate with time) can only be described by highly intricate formulas which, by chance, result in the linear relationship mentioned. By and large, it is mainly the slower reaction that determines renneting time, and which is actually the slower reaction depends on conditions.

The temperature especially affects the rate of flocculation. Consequently, when milk of low temperature (say, 5°C) is provided with rennet, κ-casein is split but the micelles fail to flocculate. When the milk is subsequently heated it clots very fast. The increase of the renneting time at still higher temperature (Fig. 21.2) is due to ongoing inactivation of the enzyme.

Milk shows considerable variation in renneting time. This especially applies to the milk of individual cows. The variation is partly caused by variation in casein content, i.e., if it is high, clotting is faster. The main parameter, however, is the Ca^{2+} activity. If it is low, flocculation is slow. The reaction can be accelerated by the addition of $CaCl_2$. Increasing the amount of $CaCl_2$ to more than a given level does not result in much change because the enzyme reaction will now determine the reaction velocity.

The influence of the pH is somewhat complicated. It appreciably affects the enzyme reaction but hardly affects the flocculation rate of the paracasein micelles, provided that the Ca^{2+} activity is not too low. At low pH the hairs are not removed at random (see Section 21.3.2) and at a similar percentage of κ-casein split fairly large bare patches will already exist on the micelles. This implies that the micelles start to flocculate at an earlier stage of the enzyme reaction. This effect explains much of the dependence of clotting time on pH. It also causes curves such as in Figure 21.3 to be different at low pH.

21.3.5 Clotting of Heated Milk

Heating milk more intensely than low pasteurization causes an increased clotting time, a weaker curd, and an impaired syneresis. The renneting time increases steeply with the heating intensity. The rate of the enzyme reaction is little affected (at most by 20%), but the flocculation becomes much slower. Addition of a little $CaCl_2$ or lowering of the pH restores the clotting time if the heating was not too intense. All the same, the effect of heating must not primarily be explained by a decrease of the Ca^{2+} activity. This is because the heating does not change the

clotting time if no β-lactoglobulin is present. The reduced rennetability and the reaction of this protein with the casein micelles (see Section 6.2) run closely parallel. Apparently, the stability of the paracasein micelles is considerably increased by the associated layer of serum protein. The detrimental effect of heat treatment can be partly undone by lowering the pH to <6 and subsequently increasing it again, e.g., to 6.4.

21.3.6   Gel Formation and Syneresis

Figure 21.3 illustrates that a gel is formed when the viscosity approaches infinity, which implies that the flocculation has advanced so far that the flocs occupy the whole volume. The modulus of the gel increases, at first because more micelles are joined together, later because the junctions between any two micelles become stronger due to fusion (Section 3.2.3). The gel is a particle gel, its properties differing considerably from those of a gel built of long macromolecules with some crosslinks. It consists of strands of casein particles, often being about three particles in thickness and some 10 particles long, alternated by some thicker nodes of particles. The gel thus contains pores, mostly several micrometers in width. The network is very irregular. Such a gel also forms by acidification of milk, but the properties involved are fairly different. As is discussed in Section 3.2 (Fig. 3.15), the properties of casein micelles depend greatly on the pH. The properties of the gels more or less reflect this effect. At pH 5.25 the bonds in the micelles are weakest, and the modulus of the gel then is lowest. At a lower temperature the bonds between the particles of the gel are stronger or their numbers are greater. Presumably, this is because the particles are more swollen and are thereby connected to each other over a larger area.

The gel tends to exhibit syneresis, i.e., contract, and to expel liquid, i.e., whey. The pores between the particles are sufficiently wide to allow streaming of liquid through them; hence syneresis. The cause of syneresis is that a particle can, in principle, form bonds with many other particles (resulting in a gain of bond energy), which would lead to a much more compact packing of the particles. This is because the particles have reactive sites all over their surface. However, they mostly cannot reach each other because they are retained in the gel network. Local breaking of bonds, which causes strands in the network to break, can lead to the subsequent formation of new and often more bonds, thereby inducing syneresis. Obviously, the tendency for syneresis will depend on conditions, and syneresis is enhanced by bonds being readily broken. Figure 21.5 illustrates the effect of pH: Near pH 5.25 the tendency to show syneresis is strongest, and it is weakest near the isoelectric pH of casein. Considerable syneresis thus can occur in cheese making, and it will increase with decreasing pH. The tendency for syneresis also considerably increases with the temperature (see also Fig. 22.7 and 22.9).

Syneresis can be affected in many ways and it is the prime variable de-

syneresis
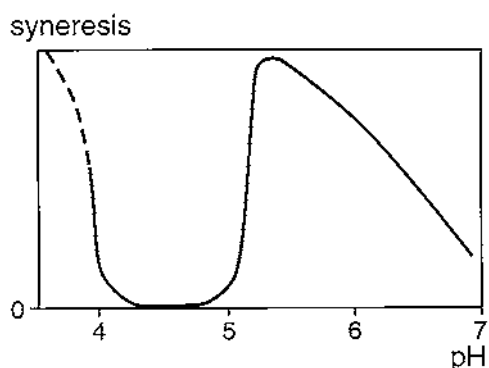
Figure 21.5 Relative syneresis rate of an acid- and/or rennet-induced milk gel as a function of the pH, at about 30°C. Approximate results.

termining the final moisture content of the cheese. This is discussed further in Section 22.2.

21.4 CHEMICAL CHANGES

In Section 21.3 physical changes are primarily discussed, though these changes ultimately result from an enzymatic reaction. Also, the latter steps in cheese making are predominantly of a physical nature, but they induce several biochemical reactions and changes in chemical composition, for the most part due to enzymes and microorganisms. Figure 21.6 illustrates in a highly schematic way the major changes that occur. The scheme is greatly simplified and fully applies for some types of cheese only; for instance, for Cheddar types, shaping and salting would occur in reverse order, and the time scale would be somewhat different. Nevertheless, the essential changes are given.

Lactic acid bacteria (predominantly *Lactococcus* species), often added in the form of a starter, play a key role. Both their metabolism and their application in the form of starters are discussed in Chapter 11. The prime action is the conversion of lactose to lactic acid, which lowers pH to, say, 5.1. An important consequence is the dissolution of the colloidal calcium phosphate, as discussed in Section 2.2.4 (see especially Fig. 2.7). This greatly affects cheese composition, the rate of syneresis, and the fusion of curd grains into a continuous mass. Moreover, some flavor components and CO_2 may be formed, and the redox potential (E_h) of the cheese will be greatly reduced. In most types of cheese, no sugar is left. Some starter organisms also produce antibiotics. All of these factors—the high concentration of lactic acid combined with a low pH; the low E_h, i.e., strictly

Time scale (h)	Physical changes	Process operations	Chemical changes
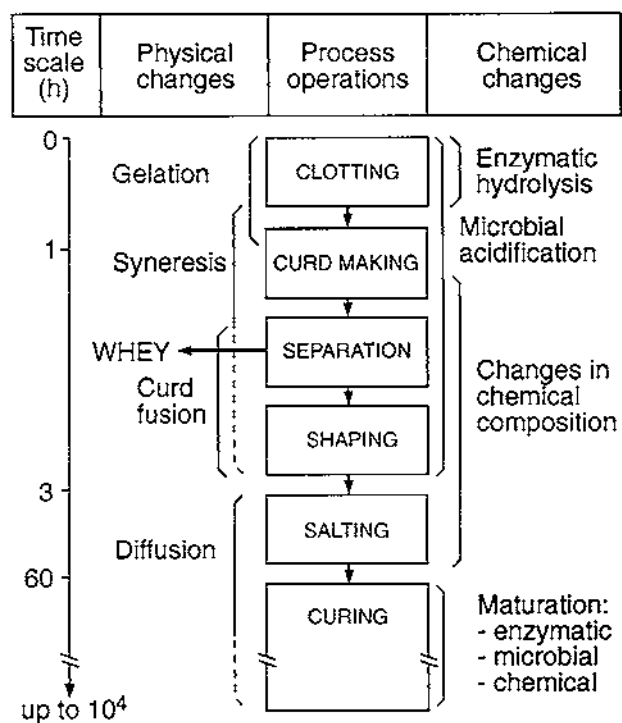
FIGURE 21.6 Scheme of the most essential physical and (bio)chemical changes occurring during the transformation of milk into cheese. Simplified example; the time scale is not linear.

anaerobic conditions; the absence of a suitable carbon source (sugar) for most bacteria; and the possible presence of other inhibiting substances—are of great importance for limiting the growth of other microorganisms, and thus for the preservation of the cheese.

The formation of a closed rind around the cheese loaf, caused by fusion of curd particles and enhanced by local moisture loss, also protects the interior of the cheese against contamination by microbes and oxygen. Salting causes another important change in composition, which further limits microbial growth (preservation). The salt also affects flavor, texture, and ripening processes.

What happens during curing may be the most complicated and thereby the least understood part of cheese manufacture. Several enzymes, originating from the milk (e.g., plasmin, lipase), or added (especially chymosin), or from microorganisms (be it starter bacteria or a more specific flora), cause a wide range of

biochemical reactions, often followed or accompanied by chemical transformations. The main changes are that a considerable part of the protein is broken down, resulting in peptides of various size, free amino acids, and smaller breakdown products. A small part of the fatty acids is split off the triglycerides. All of this is treated in Chapter 23. Moreover, diffusion of salt, water, and of the products resulting from the mentioned reactions occurs, especially from the rind to the interior and vice versa. In several types of cheese, a flora of yeasts and bacteria, and often molds as well, grow at the cheese rind, considerably altering cheese composition, e.g., by consuming lactic acid and by producing flavor compounds.

The various process steps are mutually dependent, and any variation in process conditions (temperature, amount of starter added, time allowed for process steps, and so on), influences several changes, not just one. The reactions themselves affect other reactions. Also, composition and pretreatment of the milk influence the results of process steps and ripening. All together, cheese manufacture and curing is highly intricate, and changing one factor always has several consequences. On the other hand, this allows making of cheese in a great number of variations.

SUGGESTED LITERATURE

* The best overview of fundamental aspects of cheese manufacture and properties is the book:

    P.F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology*, Vol. 1, *General Aspects*, 2nd ed., Chapman and Hall, London, 1993. Especially Chapters 2 (clotting enzymes), 3 (rennet coagulation), 4 (gel formation) and part of 5 (syneresis) relate to the present chapter.
* Another general book that contains much practical information is:

    A. Eck, ed., *Cheese Making: Science and Technology*, Lavoisier, New York, 1987.

22

Process Steps

22.1 PRETREATMENT OF MILK

22.1.1 Raw Milk

The treatments to be applied to the milk before curd making depend on the properties the cheese milk should have. These are widely variable and depend on the type of cheese produced. The following are general aspects.

22.1.1.1 Chemical Composition

This has considerable effect on yield and composition of the cheese. The milk may vary considerably in composition, especially if it originates from only a few cows as in farmhouse cheese making, or with the season if most cows calve at about the same time. However, cheese of satisfactory quality can be made of almost all milk, providing that the manufacturing process is adjusted. In most cases, the milk is standardized so as to yield the desired fat content in the dry matter of the cheese. The following are important aspects of the composition of the milk.

    a. Casein and fat content largely determine the yield of cheese. Often, the crude protein content is considered. The ratio of casein N to total N is, however, somewhat variable and the serum proteins and NPN components are hardly or not retained in the cheese.

    b. The ratio of fat to casein mainly determines the fat content in the dry matter of the cheese. It also slightly affects syneresis and thereby the ultimate water content of the cheese.

    c. The lactose content, actually the lactose content of the fat- and casein-free milk, determines the potential lactic acid production and there-

    by markedly affects pH, water content, and other properties of the cheese.

d.   The pH of the cheese also depends on the buffering capacity of the dry matter. The only important variable is the ratio of colloidal calcium phosphate to casein. This ratio does not vary greatly; generally, it increases somewhat with the stage of lactation. (For Emmentaler-type cheeses it can sometimes be too low to arrive at a satisfactory cheese quality.)

e.   The rennetability of the milk and its ability to show syneresis may vary widely, mainly because of varying Ca^{2+} activity, but other components may have an effect as well.

f.   Milk of cows suffering from severe mastitis has a low lactose content and a low ratio of casein N to total N (see also Figure 1.6); often, it clots slowly, with the curd exhibiting poor syneresis.

g.   Factors inhibiting bacterial growth may slow down the lactic acid production. Most natural factors (especially the lactoperoxidase system) do not widely vary in bulk milk. The presence of antibiotics can, however, be detrimental to acid production and maturation.

h.   The milk should not be spoiled, e.g., be rancid or show other flavor defects. However, slightly sour milk may readily be made into cheese, at least certain types of (soft) cheese made from raw milk.

22.1.1.2 Microbial Composition

The requirements involved may vary widely. For cheese made of raw milk, coliforms and propionic acid bacteria are often harmful (Chapter 24). Certain lactic acid bacteria can also cause flavor defects, e.g., yeast-like or cabbage-type flavors, whereas fecal streptococci may cause H_2S flavor. Especially high counts of lactobacilli and/or pediococci are harmful to the flavor development in hard and semihard cheeses. Although most cheese milk is pasteurized, spores of *Clostridium tyrobutyricum* may be catastrophic for many cheese varieties, e.g., Gouda and Emmentaler (see Chapter 24). Heat-resistant lipases, originating from psychrotrophs, may cause undesirable rancid or soapy flavors in many cheese types.

22.1.2 Milk Treatment

Following are some important aspects of pretreatment:

a.   *Thermalizing*, e.g., heating for 20 s at 65°C, if the milk is to be kept cool for some time. This is aimed at preventing the formation of considerable amounts of heat-resistant lipases and proteinases, and it may also reduce the count of some of the detrimental bacteria mentioned in Section 22.1.1.

b. *Removal of dirt* particles by filtering or centrifuging.
c. *Standardization* of the milk, which means adjustment of its fat content. This is discussed in Section 22.6.
d. *Pasteurization*, sufficient to inactivate alkaline phosphatase. It serves to kill pathogenic and harmful organisms (see also Fig. 22.1). A more intense pasteurization causes part of the serum proteins to become insoluble, leading to an increase in cheese yield; it decreases the rennetability and the syneresis (see Fig. 22.9); it inactivates xanthine oxidase, thereby increasing the risk of bacterial spoilage; it may increase the risk of bitter flavor development. If a heat exchanger is operating for a long time without interruption (to thermalize or pasteurize the milk) growth of *Streptococcus thermophilus* may occur in it; eventually, large numbers develop, causing flavor defects (e.g., putrid and yeasty flavors) and sometimes excessive gas formation in the cheese.
e. *Bactofugation*, sometimes applied to reduce the number of spores of *Clostridium tyrobutyricum* (to about 3%). The removal of the sediment
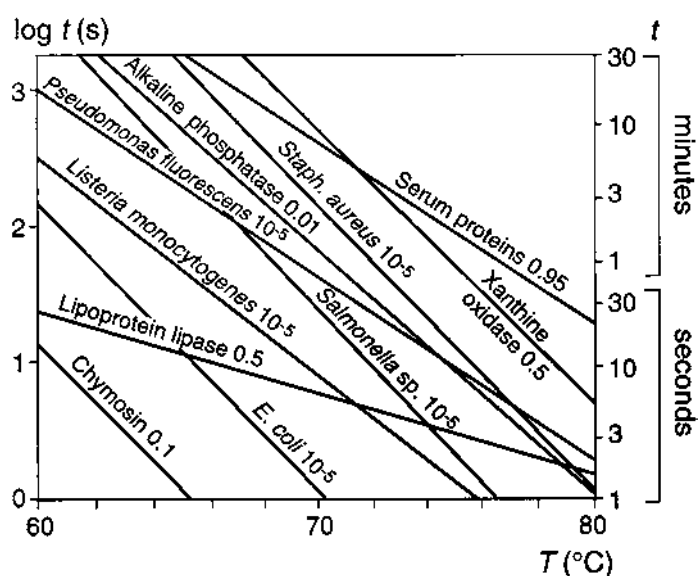
FIGURE 22.1 Time (t) needed at varying temperature (T) to inactivate some enzymes, to kill some bacteria, and to denature serum proteins in milk or whey (at pH 6.7). The figures indicate the approximate fraction left unchanged after the treatment.

obtained, containing the spores, causes about 6% reduction in cheese yield. Therefore, the sediment is UHT-heated and added again to the cheese milk.

f. *Cold storage* of the milk. Before rennet addition, the cold-stored milk should be kept for some time at renneting temperature or, rather, be briefly preheated to, say, 50°C, to improve the rennetability. The milk is often pasteurized immediately before the renneting.

g. Prevention of *recontamination*; see, for instance, Chapter 24.

h. Prevention of *damage to fat globules*, which must be considered in connection with potential lipase action and with ''churning,'' i.e., the formation of visible lumps of fat. Such damage may occur when air is beaten in, especially if the milk is splashed from a height into the cheese vat. Warming the cold-stored milk sufficiently to melt the fat and then cooling it to renneting temperature prevents churning.

i. *Homogenization* of the milk causes a different, in some cheeses an undesirable sticky, texture and additional lipolysis. The latter may be desirable for blue-veined cheese.

j. *Addition of substances*, including

- Starter.
- Calcium chloride. This speeds up the clotting or reduces the amount of rennet needed, and leads to a firmer gel; it especially diminishes the natural variation in rennetability.
- Potassium (or sodium) nitrate, if desired, to suppress fermentation of butyric acid and coliform bacteria (Chapter 24).
- Sometimes coloring matter (either annatto or carotene).

22.2 CURD MAKING

22.2.1 Concentrating the Protein

The main objective of the first stages of cheese making is to collect the protein, enclosing most of the fat, whereas most other constituents, including water, should largely be removed. The composition should become such that a coherent mass can be obtained, exhibiting the desirable consistency, flavor, and keeping quality after further treatment and maturation. An additional and important objective is that the process be brief, effective, and economical; it should be well controllable and cause little loss of curd and fat. For the sake of efficiency, continuous processing would be preferable. However, due to the numerous problems that may occur with continuous manufacture, which then lead to considerable loss of milk or curd, batch processing is usually applied, at least for hard and semihard cheeses.

In principle, two courses are open: first insolubilizing the protein and concentrating it subsequently, or the other way around. We will first discuss examples of the former method.

22.2.1.1 Rennet Coagulation

This implies clotting as influenced by a proteolytic enzyme. It can be a vegetable enzyme extracted from fig leaves (*Ficus*), certain thistles (*Carduus*), *Bromelia* species, etc. Nowadays, enzymes originating from microorganisms are sometimes applied. In Western countries rennet from calf stomachs is commonly used; chymosin and pepsin (e.g., ratio of 4:1) are the active components (see also Section 23.3). By means of DNA recombination microorganisms can be ''engineered'' to produce pure bovine chymosin.

To compare the activity of various rennets, the *rennet strength* can be determined. Generally, a particular rennet is standardized so as to yield a desired strength, e.g., 10 000 Soxhlet units. It means that 1 g of the rennet involved can cause a beginning coagulation of 10 000 g of normal fresh milk at 35°C in 40 min. *Clotting time* is defined below; for *rennetability* of milk, see Section 22.2.1.6.

The physical chemistry of the rennet coagulation of milk is discussed in Section 21.3. As is shown in Figures 21.3 and 21.4, most of the κ-casein must have been split before appreciable aggregation of (para)casein micelles occurs. The paracasein micelles flocculate to form irregular, often somewhat thread-like aggregates that finally form a continuous network, hence a gel (see Section 3.2 and Figure 22.2). The gel rapidly increases in firmness and at a certain moment is firm enough to be cut into cubes. The cutting is necessary to allow rapid expulsion of whey (Section 22.2.2). In Figure 22.3 the shear modulus is given as a function of the time after rennet addition. At cutting, the modulus is just a small percentage of the value attained when the gel is left undisturbed for some hours. Initially, the modulus increases as the number of contacts between micelles increases (see Fig. 22.2). Later on, a strengthening of the bonds between the mi-
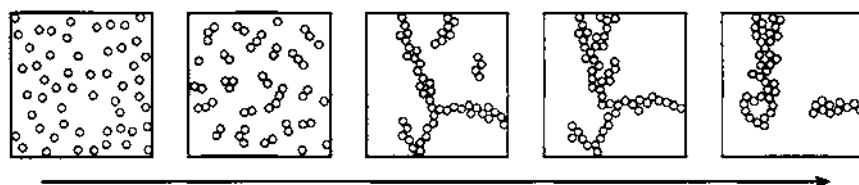
FIGURE 22.2 Representation of the flocculation of paracasein micelles, the formation of a gel, and the start of (micro)syneresis. Highly schematic.
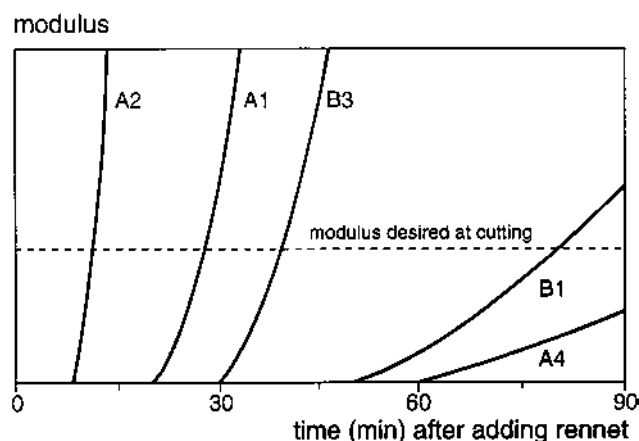
modulus

FIGURE 22.3 Firmness (expressed as shear modulus) of renneted milk as a function of time. Two milks: A is a rather average mixed milk, B a very slowly renneting milk of one cow. Renneting at 30°C, 0.03% rennet. Further conditions: 1 = unchanged milk, 2 = lowered pH (6.4), 3 = $CaCl_2$ added (1 mM), 4 = heated (15 s at 95°C). Approximate examples.

celles (because the contact area between any two micelles increases) accounts for the increased modulus.

Several factors affect the clotting rate of milk (see Fig. 21.2), and, as is shown in Figure 22.3, factors causing a decrease of the renneting time also cause a faster increase of the gel firmness. Naturally, the rate of clotting depends on the amount of rennet added. According to the rule of Storch and Segelcke, the flocculation time is inversely proportional to the rennet concentration. The flocculation time is defined here as the time between the addition of rennet and the presence of the first flocs as visually observed. Of greater interest is the time needed for the gel to become firm enough to be cut, often called the clotting time (Fig. 22.4). The rule of Storch and Segelcke does not apply very well to the latter. The clotting time closely depends on the temperature. At about 30°C it decreases by some 10% for every degree Celsius of temperature increase. Usually, the milk is renneted at about 30°C, while so much rennet is added (e.g., 0.025% with a specific activity of 11 000 Soxhlet units) that the milk clots in 20–30 min. Both lowering the pH and adding $CaCl_2$ speed up the clotting (Fig. 22.3).

A uniform gel is only obtained if the milk is at rest during clotting. Because of this, it is difficult to perform the clotting as a continuous process. It is, however, possible by means of the method of Berridge, in which milk of low temperature
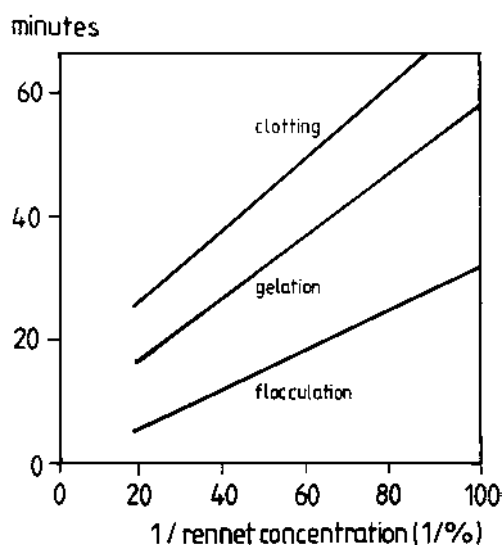
FIGURE 22.4 Time needed for visible flocculation, to start gelation and to obtain sufficient firmness of the gel (clotting time), as a function of the rennet concentration. Approximate examples (30°C) for low-pasteurized milk; CaCl$_2$ is not added. After A.C.M. van Hooydonk and G. van den Berg, Bulletin of the IDF 225 (1988) 2.

(say, 5°C) is provided with rennet. Although the enzyme does work (be it more slowly than at 30°C), the micelles fail to flocculate (see Fig. 21.2). After the κ-casein has been split, the milk is fast-heated to about 30°C, and now it clots in a very short time (about 1 min). The process can be achieved continuously, e.g., in a wide tube in which the milk flows slowly. The gel formed can simply be cut into cubes.

A part of the rennet (say, 15%) is recovered in the cheese (see Section 22.2.1.5). This greatly affects the ripening, i.e., the protein degradation (see Section 23.3). In the manufacture of some cheese varieties (Emmentaler, some of the Italian cheeses) heating during the curd treatment (cooking or scalding) is sufficient to inactivate a considerable proportion of the rennet (see also Figs. 21.2 and 22.1). The inactivation is much stronger at a relatively high pH (e.g., 6.4) than at a lower one (e.g., pH 5.3).

22.2.1.2 Acid Coagulation

Milk can be clotted by lowering the pH. This is mostly effected by lactic acid bacteria. Possibly, proteolytic enzymes of the bacteria also play a part because

some of those can split κ-casein. However, the main mechanism is casein becoming insoluble near its isoelectric pH.

If the temperature of acid production is not too low (e.g., 30°C) and the milk is at rest, a gel forms, similar to that at rennet coagulation. However, the gel is rather different. For instance, it is shorter and may be firmer than a rennet-induced gel. Its composition also differs. Rennet coagulation yields a coagulum of paracasein micelles including colloidal calcium phosphate, whereas an acid milk gel consists of casein particles of a similar size as in rennet coagulation, but without calcium phosphate. The acid gel can be cut and stirred like a rennet gel (Section 22.2.2), but it shows little syneresis, especially in the pH range 4.2–5. For removal of a sufficient amount of whey the temperature should be fairly high, but low-moisture cheese cannot be made via acid coagulation.

Alternatively, the milk may be acidified at some lower temperature while stirring, so that a voluminous precipitate forms, not a gel. Centrifugation separates the soured milk into a rather smooth, pumpable curd and whey. The dry matter content of such curd cannot be made higher than 23%, or about 17% if skim milk is used. Therefore, the latter method is mainly applied in the manufacture of quarg; a fully continuous process can be applied.

Acid coagulation by bacterial growth requires a long time, even at optimum temperature. Of course, acid can also be added directly, for instance lactic acid or hydrochloric acid. This causes the milk to coagulate during acidification and gives a very irregular curd. Instead of acid, a lactone can be added, which subsequently is slowly hydrolyzed (i.e., for several minutes) to form an acid. In this way a homogeneous gel can be made. Finally, milk at a low temperature (about 5°C) can be supplied with acid, obviously not by means of bacteria. Just as with rennet coagulation, acid coagulation does not occur at low temperature. After acidification, the milk is uniformly heated. Again, this way of working is based on the above-mentioned principle of Berridge. It is used in the manufacture of cottage cheese (Section 25.2).

Often, a combined acid and rennet coagulation is applied, especially in the manufacture of fresh and ripened soft cheeses. In fact, rennet coagulation often is more or less enhanced by acid coagulation, since normally starter is added. Further, the manufacture of some cheeses includes preculturing of the milk before rennet addition. The lower the pH, the faster the clotting (quantity of rennet being equal) (see Fig. 21.2). This is not so much due to the pH itself as to the increased calcium ion activity. Syneresis also is faster.

22.2.1.3   Coagulation by Heating

Heating of milk per se does not cause clotting or coagulation. To be sure, a considerable proportion of the serum proteins becomes insoluble, depending on both time and temperature of heating (Sections 2.4 and 6.2). These proteins largely associate with the casein micelles and, consequently, are recovered in the

cheese after coagulation with rennet or acid and syneresis. This may cause problems. The milk clots less satisfactorily with rennet (it takes a long time before a gel forms which, moreover, is not very firm) or even fails to clot (see Fig. 22.3 and Section 21.3.5). The water content of the curd remains high, even after extended curd treatment. Usually, during maturation bitter compounds form. Often, an increase of cheese yield by heat treatment of the milk is undesirable.

Heating can be applied to recover lactalbumin from whey (Chapter 18). The protein is then added to cheese milk, i.e., in the Centri-whey process as sometimes applied in soft cheese manufacture. Alternatively, a protein-rich coagulum, obtained from acid whey by means of heat denaturation, can be processed as such (including molding, salting, curing) to arrive at a protein-rich product, sometimes designated as cheese.

Furthermore, addition of lactic acid to high-heated skim milk is applied in the production of Schabziger, a ripened cheese in Switzerland; alternatively, a mixture of skim milk and whey is used. Heating of sour milk causes curdling of the previously flocculated casein, so that it can be readily separated. Formerly, the heated sour milk was drained in a cheesecloth bag, yielding a primitive type of quarg or bag cheese. In some tropical countries a kind of fresh cheese is made by adding acid to milk, followed by heating to a fairly high temperature.

22.2.1.4 Concentrating Before Clotting

It is conceivable first to make a liquid product similar in composition to unsalted cheese and to subsequently gel the mixture. Evaporation of the milk yields a mixture of the wrong composition. (Evaporation is applied in the Hutin-Stenne process. Briefly: evaporating, cooling, adding rennet and letting it split κ-casein, diluting with warm water causing clotting, allowing syneresis, drainage. All of these steps proceed continuously. Applied for soft cheeses.) Accumulation of casein micelles by means of centrifugation or gel filtration is too expensive.

What is indeed applied is *ultrafiltration* (see Section 9.4.1). The process of manufacture has been developed predominantly in a French dairy research institute. In principle it runs as follows: Milk is separated, skim milk is ultrafiltered, water may be added to the concentrate and the milk again ultrafiltered (diafiltration), concentrate and cream are blended in such a way as to approach the composition of the unsalted cheese, starter and rennet are added, the mixture is renneted while flowing through a tube, slices are cut from the extruding cylinder of clotted milk, the freshly made cheese is salted and cured. Usually, a slight syneresis still occurs. In this way cheese can be made with up to a maximum of 25% solids-not-fat (at 45% fat in the dry matter). Hence, mainly soft cheeses are produced. Currently, whole milk is also ultrafiltered.

For cheese with a relatively firm consistency, the milk may be partially concentrated by ultrafiltration, followed by renneting, cutting, and a gentle curd treatment. The benefits are obvious:

1. A slightly higher yield is obtained because a proportion of the serum proteins is incorporated into the cheese (if the concentration factor is above 3).
2. There are savings on rennet and other additives.
3. There is more efficient use of the curd-making equipment when a traditional method of manufacture is applied.
4. To a certain extent, and for soft cheese a great extent, automated continuous processes can be applied.

However, the method is hard to perform and continuous manufacture necessitates expensive machinery. The quality of the cheese is unsatisfactory; maturation is slower, the flavor usually remains somewhat flat, and the cheese is relatively soft. The latter factor may be of advantage in the production of low-fat cheese.

22.2.1.5   Accumulation of Other Constituents

Due to clotting a continuous network forms consisting of protein particles, usually paracasein micelles. The pores of the network are of the order of a few micrometers in width. Initially, the network encloses all of the milk, but it soon starts to contract, i.e., to show syneresis. This is illustrated in Figure 22.2. Thereby the moisture (i.e., water + dissolved substances) is squeezed out. With respect to dissolved substances this implies that at the moment of drainage the composition of the moisture in the curd is similar to that in the whey. On closer inspection, the ratio of water to dissolved substances in the curd is higher, as is discussed in Section 9.1. A certain quantity of nonsolvent water can be defined, which is larger for solute molecules of larger size. For the serum proteins in milk, the quantity of nonsolvent water is about 2 to 3 g per g paracasein. Most cheese contains approximately this amount of water or less. It is indeed observed that semihard and hard cheeses are virtually devoid of serum proteins (and probably also of the caseinomacropeptide split off κ-casein by rennet). This would also imply that enzymes that occur dissolved in the serum of milk would not reach the cheese. Cheese varieties of a high water content may contain some serum proteins and serum enzymes. Also, cheese made via ultrafiltration of the cheese milk will contain these proteins.

On the other hand, some proteins, notably enzymes, tend to adsorb onto paracasein micelles, and these will accumulate in the cheese. This concerns the indigenous proteolytic enzymes plasmin and acid milk protease. It also occurs with chymosin and pepsin, and this is paramount for cheese ripening (Section 23.3). The adsorption of chymosin is virtually nil at pH 6.7, but it greatly increases with decreasing pH. The amount of chymosin (and pepsin) retained in the curd, and thereby reaching the cheese, thus depends on conditions during curd making, especially the pH at the drainage state. The amount retained in the

cheese varies from 1% to 40% of the quantity added to the milk. This is one of the most important variables determining the rate of proteolysis in cheese. Enzymes that are added to cheese milk may also adsorb onto the paracasein micelles. An example is lysozyme, which adsorbs strongly, and which is sometimes added to inhibit growth of butyric acid bacteria (see Section 24.2).

Enzymes that are in the membrane of fat globules are also incorporated into the cheese; this primarily applies to xanthine oxidase and various aminotransferases and phosphatases. This is because all of the particles in the milk, i.e., fat globules, microorganisms, somatic cells, and dirt particles, are for the greater part mechanically entrapped in the network of aggregated paracasein micelles. The number of starter bacteria per gram of material, for instance, is about 10 times higher in cheese than in the cheese milk, not considering any growth.

Most important for the cheese composition is that not only protein is accumulated but also fat. Treatment of the curd, i.e., cutting and stirring, causes loss of particles, especially at the cut surfaces. For instance, about 6% of the fat is lost with the whey. Most of this fat is recovered by centrifugation of the whey. A part of the curd, defined as curd fines, is also lost in the whey. Losses of fat and fines greatly depend on the firmness of the renneted milk at the moment of cutting and on the intensity of cutting and stirring. If the curd is too weak, the losses are great; if it is too firm, cutting needs excessive force.

If the particles are not only entrapped in the network, but attach to it, or even form part of it, their loss during the mechanical curd treatment is lower. Fat globules become part of the casein network if they have casein in their surface layer. This can occur because of damage during processing of the milk (beating in of air, evaporation, atomization) and especially by homogenization. In addition to a smaller loss of fat into the whey, a somewhat different consistency of the cheese results, often designated as ''sticky.''

22.2.1.6 Variability

The manner in which the curd making proceeds, and thereby the composition of the cheese, vary widely, especially with milk composition if the manufacturing conditions are not adjusted. There are several variables.

The ratios of the concentrations of the principal constituents of the milk vary and so does the composition of the cheese. The fat content can be standardized relative to the casein content. The lactose content of the milk affects the pH of the cheese; accordingly, the curd making will often be adapted to arrive at the desired pH (see Section 22.2.2).

The composition of the milk affects rennetability, firmness of the curd, and syneresis. The rennetability, expressed as the quantity of milk that is clotted by a certain amount of rennet (enzyme) in a certain time and at a certain temperature, can vary by a factor of approximately 2 in mixed milk. Lactation stage and differences among cows appear to be the main sources of variation. In practice, there-

fore, season and area of milk supply are important variables. The manufacture thus will have to be adapted, e.g., renneting temperature, rennet concentration, added $CaCl_2$, clotting time. Adding a fixed amount of $CaCl_2$ considerably reduces the variation, though small variations persist.

Milk of other animal species, e.g., sheep, goats, and buffaloes, behaves differently from cows' milk if used for cheese making. Ewes' milk has a lactose content comparable to that of cows' milk but its fat, protein, and total solids content are distinctly higher (Table 1.5). Accordingly, under similar conditions it clots to a very firm coagulum.

The pretreatment of the milk can have an effect. Factors are:

- Pasteurization (see Section 22.2.1.3)
- Cold storage diminishes rennetability (Section 22.1.2)
- The amount of starter added and the time allowed to produce lactic acid before renneting determine the pH and therefore the rennetability.

Changes during curd treatment are discussed in Section 22.2.2.

22.2.2   Curd Treatment in the Vat

Here we will consider curd formed by rennet coagulation. The subsequent curd treatment, including cutting, stirring, cooking (scalding), and drainage, mainly determines the composition of the cheese. Of special importance are the water content and the pH. The characteristic and most important phenomenon involved is syneresis. Acid production and washing (i.e., dilution of whey + curd with water) also play an important part. The effect of cheddaring is discussed in Section 25.5.1. (See also Section 21.3.)

22.2.2.1   What Is Syneresis?

The rennet gel tends to contract soon after it has formed. This cannot be ascribed to a persisting action of rennet, since no additional caseinomacropeptide is removed from the micelles shortly after the gel has formed. This means that the number of reactive sites on the paracasein micelles does not increase. However, the flocculated micelles do not have a close-packed arrangement; the gel largely consists of strands of micelles that join together locally. Therefore, far more bonds between micelles can form (see also Fig. 22.2). This proceeds fairly slowly since formation of these bonds is geometrically difficult; the casein particles are all incorporated in the network and thus have very little freedom of motion. To have a substantial syneresis some bonds should first be loosened, i.e., strands of micelles in the network must break, to allow formation of more new bonds.

The shrinkage of the gel proceeds only slowly, mainly because the whey should flow through the meshes of the gel network. According to Darcy, the

superficial velocity v of a liquid of viscosity η flowing through a porous material is given by:

$$v \equiv \frac{Q}{A} = B \, \Delta p / \eta l \qquad (22.1)$$

where Q is the volume flow through a cross-sectional area A. Initially, the permeability coefficient B of a rennet milk gel is approximately 0.2 μm^2. The greater l (l being the distance over which the liquid has to flow), the greater the relative drag. Hence, making l small has a beneficial effect. It is achieved by cutting the gel into cubes. Cutting also greatly increases the area through which the whey can flow out; after all, it is the volume flow rate $Q = vA$ in which we are interested.

The endogenous syneresis pressure Δp (the ''driving force'') turns out to be exceptionally small, i.e., about 1 Pa (corresponding to a column of water of 0.1 mm height). Accordingly, it is normal practice to enhance the syneresis by exerting mechanical pressure, which has a considerable effect (see Fig. 22.5). Presumably, the syneresis is not only enhanced by the external pressure itself but by the resultant deformation of the curd grains, since such deformation causes breaking of strands, thereby enhancing formation of new bonds between micelles.

At first, and as long as the external conditions do not change, the quantity of moisture removed is almost proportional to the curd surface area and to the
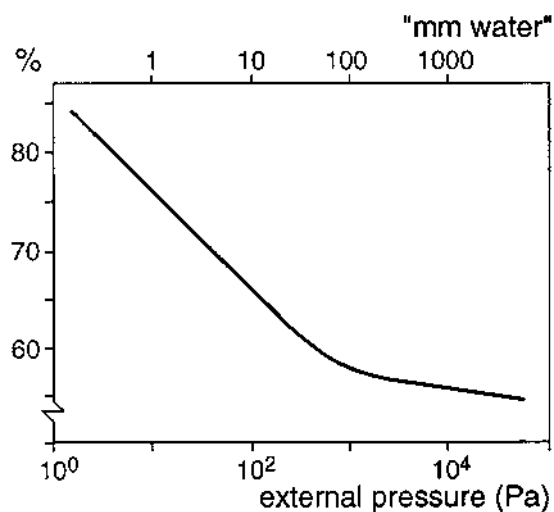
FIGURE 22.5 Water content (%) of curd made of whole milk after 1 h stirring and 1 h pressing under the whey at various pressures. pH and temperature were kept constant.

square root of time, i.e., as in a diffusion process (of course, syneresis is not diffusion!). Accordingly, the syneresis proceeds ever more slowly. Furthermore, at the start of the process large quantities of whey must be expelled to achieve a certain decrease of the water content of the curd, whereas at the end a small loss of whey corresponds to a marked decrease (see Fig. 22.6).

The course of the syneresis is fairly intricate because both the endogenous syneresis pressure and the permeability change with time and with continuing concentration due to syneresis (see Fig. 22.7). If no whey is expelled, the permeability of the gel increases during its keeping. This should be ascribed to microsyneresis, which implies that in some spots the network of paracasein micelles will become more dense, whereas wider pores are formed on adjacent sites. If, however, whey can drain off, the whole network is condensed and the permeability decreases. Initially, the syneresis pressure increases as a result of an increased formation of reactive sites in the network. Later on it decreases because the gel becomes firmer due to stronger bonds, causing strands of micelles to break less readily.

Eventually, the syneresis becomes slower and slower. After some time it should stop because the gel cannot shrink any further. It then has attained its most close-packed arrangement of casein micelles and fat globules. Consequently, the
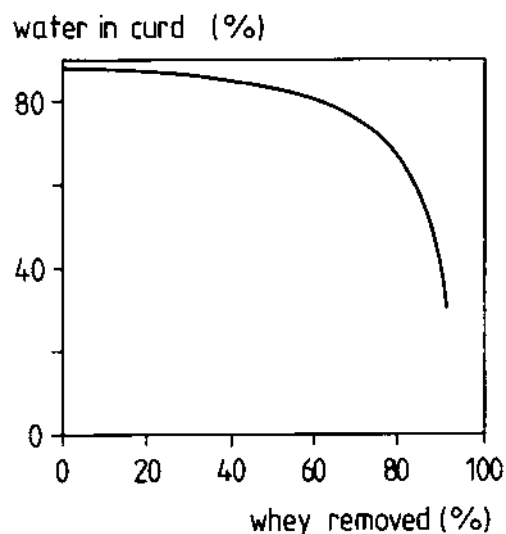
FIGURE 22.6 Calculated relation between the water content of curd (of whole milk of 87.7% water) and the quantity of whey (6.8% total solids) expelled as a percentage (w/w) of the original milk.
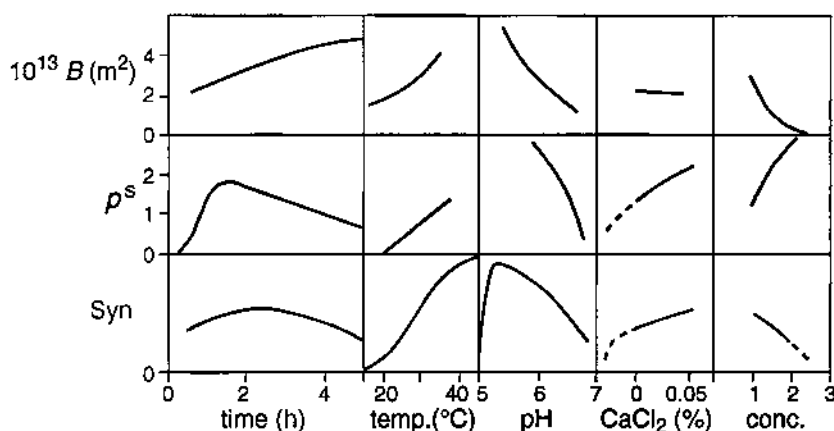
FIGURE 22.7 Effect of time after rennet addition, temperature, pH, added CaCl₂, and concentrating of the milk (by ultrafiltration) on syneresis: permeability (B), endogenous syneresis pressure (p^s), and approximate syneresis rate (Syn). The influence of the time refers to situations without (macro)syneresis, i.e., the syneresis is allowed to begin at the time indicated. Note that during practical curd treatment syneresis does occur (at least locally, at the outer layer of the curd grains); this implies that the network is condensed, which may lead to quite different relationships, such as a (marked) decrease of B.

syneresis rate should be distinguished from the total syneresis, which determines the final water content.

Naturally, syneresis proceeds fastest at the outer rind of a cube of curd, where the whey can readily drain. Here, in turn, a dense layer forms that has a low permeability coefficient B and retards syneresis. The layer may be called a ''skin'' if it is very dense.

22.2.2.2 Factors Affecting Syneresis

The effect that some factors have on syneresis may vary widely according to conditions. The main cause is that syneresis pressure as well as permeability can be affected, as can be seen in Fig. 22.7. If one desires to make high-moisture cheese, the syneresis should be slowed down or stopped after a certain time. The rate of syneresis then considerably affects the final result. However, the syneresis rate is far less important if cheese is made with a low water content. After all, the curd treatment can be continued until equilibrium has been nearly attained. (For practical reasons, the time required will mostly be kept as short as possible.)

a. *Firmness of the gel at cutting*. If the gel is still weak at cutting it tends to synerese slightly, but syneresis tends to increase rapidly. Of greater importance is that a large amount of curd fines is released, causing a reduced yield of cheese.

b. *Surface area of the curd*. Initially, the syneresis is proportional to the area of the interface between curd and whey. Accordingly, the gel expels very little whey if it is not cut and keeps sticking to the wall of the vessel. (Clotted milk sticks to most hydrophilic materials, though not to copper; it sticks more or less to stainless steel, especially if it is treated with alkali.) Therefore, the gel is often cut into cubes. The smaller the cubes, the faster the syneresis. At the same time, more fat and curd fines are transferred to the whey. If the curd granules differ widely in initial size, the resulting cheese may be very inhomogeneous. Small grains shrink fastest and hence become dryest. Moreover, most of these grains settle at the bottom of the layer of curd grains. Small differences in the average size of the curd grains have little effect on the final water content.

   In the traditional manufacture of soft-type cheeses, large lumps are cut from the coagulum and ladled into molds, where the syneresis occurs. This leads to a high water content.

   Fine curd grains are obtained, as is a dry cheese if the milk is stirred during renneting and after. Of course, the loss of curd fines and fat is also markedly increased.

c. *Pressure*. Figure 22.5 has already been discussed briefly. The result holds for curd under the whey. Stirring exerts pressure, partly because of the pressure difference due to velocity gradients in the liquid, which, according to Bernoulli's law, is $\frac{1}{2}\,\rho d^2 G^2$; ρ is density, d the diameter of the curd grain, and G the velocity gradient. This causes pressures of the order of 10 Pa. Of more importance may be that the curd grains collide and thereby compress one another for a short while. Furthermore, stirring prevents sedimentation of the curd; in a layer of curd grains the surface of the grains available to release whey rapidly diminishes, thus slowing down syneresis. Figure 22.8 gives an example of the influence of stirring. The effect markedly increases with increasing ratio of curd to whey (thus by removal of whey) and stirring rate. Stirring or working the drained curd also causes a lower water content.

d. *Acidity*. The marked effect of the pH is shown in Figure 22.7. The explanation is not yet clear. Syneresis rate thus markedly increases with decreasing pH, but it decreases sharply if the pH is decreased to below 5.1. The permeability is scarcely affected, but the endogenous syneresis pressure falls to about zero. Incidentally, it makes some difference as to whether the pH is lowered before or after the gel has
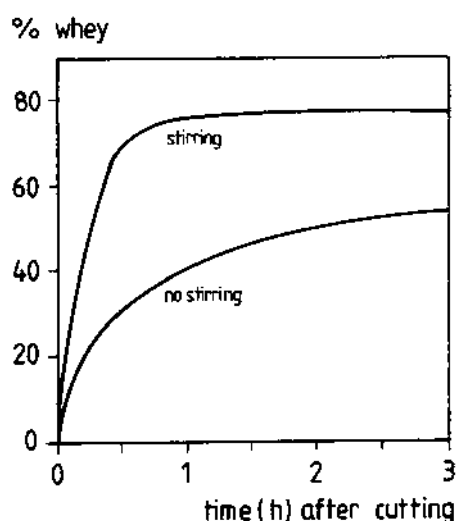
FIGURE 22.8   The volume of whey released (as percentage of the original volume of milk) as a function of time after the start of cutting, with or without stirring. Curd kept in the whey, temperature 38°C. After A. J. Lawrence, *Austr. J. Dairy Technol.* **14** (1959) 169.

formed. In practical cheese making with rennet coagulation, more acid production always implies a faster syneresis and a lower final water content.

e. *Temperature*. This has such a strong effect that syneresis can be stopped by cooling (see Fig. 22.7). Increase of temperature markedly accelerates syneresis. However, a very quick rise of temperature (e.g., by adding hot water) causes the outer layer of the curd grains to synerese so fast that a ''skin'' forms, i.e., a layer of such low permeability that it slows down any further syneresis; an additional reason may be that the skin has become so firm as to mechanically hinder further shrinkage of the curd grain.

The temperature also affects swelling and shrinkage of the paracasein matrix. It appears that as the temperature is lowered, the casein becomes more swollen (see also Fig. 3.14). This is of importance when the syneresis has almost been achieved, i.e., when the paracasein micelles have reached a rather close-packed arrangement. Increasing the temperature of such a concentrated paracasein gel may cause expulsion of moisture, whereas a drop of the temperature may lead to absorption of moisture.

    f.   *Composition of the milk*. The higher the fat content, the less the curd can shrink; hence, a higher final water content in the fat-free cheese. (The water content in the cheese is lower because there is more fat, substituting fat-free cheese.) The fat also impedes the flow of whey out of the curd, i.e., the permeability B in Eq. (22.1) is lower. Hence, the fat reduces the syneresis rate.

        Furthermore, Ca^{2+} activity, pH, protein content, and concentration of colloidal calcium phosphate affect syneresis. Most milk of cows in early lactation clots faster and shows more syneresis. There is considerable variation among milks of individual cows.

    g. *Numerous other variables* also have an effect. Several factors that can be varied in practical cheese making are summarized in Figure 22.9.

22.2.2.3 Acid Production and Washing

The pH in the curd decreases because of the action of the starter bacteria. This decrease greatly enhances the syneresis. The rate of decrease in pH is determined by factors including starter (amount added, type, strain), composition and pretreatment of the milk, and temperature during curd treatment (see Section 11.4).

    Almost all starter bacteria are entrapped in the curd, implying that the acid is mainly produced in the curd grains. As long as the grains are in the whey, lactic acid can diffuse from them into the whey and lactose in the opposite direction. In this way, acid is produced in the whole mixture. After drainage of the whey the accumulation of acid in the curd proceeds more quickly and the lactose content in the curd decreases more rapidly.

    In most cases, the rate of acid production, hence the pH at molding, has little effect on the final pH of the cheese. In most cheese types all lactose is converted, mainly to lactic acid. It implies that the ratio between lactic acid and buffering compounds determines the pH. The moisture content of the curd is paramount. The higher it is, the more lactose, or its equivalent lactic acid, is retained in the curd and the more acidic the resulting cheese will be. The main buffering substances are paracasein and calcium phosphate.

    The rate of acid production has a secondary effect on the pH of the cheese. To begin with, it can affect syneresis and thereby the moisture content of the cheese. If the latter is held constant by means of additional measures a small effect persists, e.g., minus 0.1 unit in pH. If the curd is more acidic at the moment of molding, more calcium phosphate has dissolved (Fig. 22.9; see also Fig. 22.17) and thus less buffering substance is left in the cheese. Preacidification and inoculation with a great amount of starter have similar effects. Furthermore, at identical moisture content, a lower pH at molding causes a slightly lower yield of cheese dry matter; it also affects cheese texture, with the consistency becoming softer at the same pH. If cheese is salted at the curd stage, the bulk

Variable	pH	Ca	Syn	Wff	Rennet
fat content of milk					
pasteurization intensity					?
cold storage of milk					
amount of CaCl$_2$ added					
amount of starter added					
preacidification					
renneting time[1]					
renneting temperature					
curd cube size	–				
stirring intensity					
amount of whey removed					
time until scalding			–		
scalding temperature					
amount of water added					
time until pitching[2]			–		
pressure on curd layer	–	–			
duration of pressing[3]			–		

FIGURE 22.9 Effects of pretreatment of the milk and of conditions during curd making on the pH of the curd at the end of curd making (thus not the final pH of the cheese), the amount of Ca retained in the cheese, the rate of syneresis (Syn), the final water content of the fat-free cheese (Wff), and the quantity of rennet retained in the cheese. The relations are only meant to illustrate trends for cheese of average water content. (blank) The relation is unknown but probably is weak. (dash) No relation. Notes: (1) This roughly implies the reciprocal of rennet quantity. (2) Total time between cutting and pitching. (3) On the curd layer, e.g., by means of metal plates on top (not in the molds).

of the lactose must have been converted before molding because the lactic acid fermentation is markedly slowed down by the added salt.

To adjust the pH of the cheese independently of its moisture content, other steps should be taken. A smaller drop of the pH is achieved by washing, i.e., adding water to the mixture of whey and curd. The lactose diffuses away from the curd grains until identical concentrations have been attained in the water inside and outside the curd. The effect of the washing closely depends on the size of the grains and on the contact time. Equilibrium is rarely achieved. In practice, the efficiency of reducing the lactose concentration in the curd approximates 90%.

The wash water is commonly also employed to raise the temperature of the curd and whey mixture, i.e., scalding. The higher temperature causes the syneresis to be stronger. There are also other methods for scalding the curd, such as direct heating.

22.2.2.4 Separation of Curd and Whey

Figure 22.10 illustrates that the moisture content of the curd sharply decreases when the curd is taken out of the whey. This effect should largely be ascribed
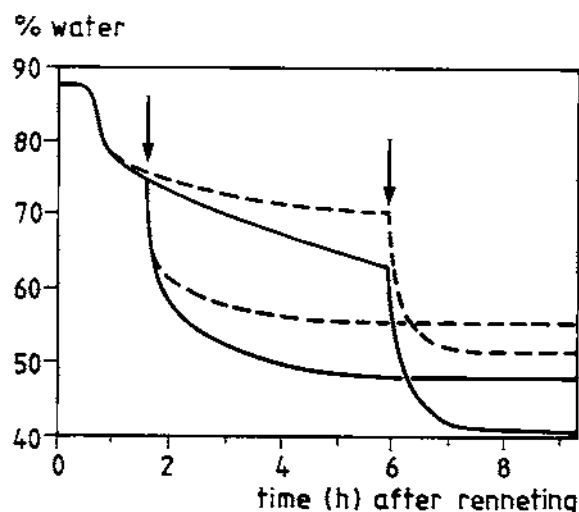
FIGURE 22.10 Examples of the course of the water content of curd during curd making. Cutting after 0.5 h. At two moments (indicated by arrows) curd was taken out of the whey and put into a cheese mold. The dotted lines refer to experiments without added starter. The curd and whey mixture was continually stirred. Temperature in the whey was 32°C throughout; temperature in the mold gradually fell to 20°C.

to a greater pressure on the curd. Further, it is seen that extended stirring causes a lower final water content. If the temperature of the curd mass could be kept constant after drainage of the whey, then the time of separation would affect the final water content only slightly. This illustrates that in practical cheese making the lowering of the temperature after the whey drainage rapidly restrains syneresis. The larger the block of curd, i.e., the larger the loaf of cheese, the slower it cools and the lower the final water content will be, although the transport distance of the moisture is longer in a larger loaf (see also Fig. 22.12).

The curd grains are usually allowed to settle and form a layer. Alternatively, the mixture of curd and whey is transferred to a vertical drainage cylinder, in which the curd settles. As long as the curd is under the whey, any mechanical pressure has an effect on the water content (Fig. 22.5), and so has the height of the column. The drainage of whey may be crucial in a column of curd in a drainage cylinder. Whey flows primarily between the curd grains, but these deform because of the pressure involved and also fuse, lowering the curd surface area, narrowing the pores between the grains and eventually almost closing them. A great spread in the size of the curd grains (e.g., presence of many curd fines) enhances the pores' becoming blocked, which decreases drainage. According to conditions one or the other process will prevail, i.e., enhancement of syneresis by pressure or slowing it down by fusion, respectively. Figure 22.11 illustrates all of these effects.

In the cheddaring process (Section 25.5), the drained curd is left for a long time, preferably without cooling it too much. Meanwhile, considerable acid production occurs. The long time and the low pH cause the moisture content to become low. However, during cheddaring the curd is allowed to spread and this enhances closing of the pores between curd grains and fusing of grains. The latter two factors cause the decrease of the moisture content to be less than it would be if the curd could not spread. The difference amounts to some 1% to 2% water in the cheese.

If the curd has been made very dry before drainage of the whey, e.g., by means of prolonged stirring or a high scalding temperature, a higher temperature during drainage leads to a higher water content. Probably, this is caused by a more rapid closing of pores between curd grains which, in turn, is due to a more rapid deformation at the higher temperature. Correspondingly, in *this* case a smaller loaf of cheese attains a lower water content.

To make curd very dry the drained curd can be stirred again, i.e., be ''crumbled.'' This causes a considerable loss of fat and curd fines in the whey and marked mechanical openness in the cheese.

It will be clear that the various process steps should be matched to one another. Figure 22.10 shows this to be difficult if the curd, after it has been made batchwise in a tank, is subsequently drained, molded, and pressed in a continuous process, as syneresis goes on in the tank. It leads to significant variation in water

Process	depends on:

1. syneresis $\longrightarrow$
   a. higher concentration
   b. curd less deformable
	concentration, temperature, pH,
	 pressure (height curd column),
	 curd surface area

2. outflow of whey $\longrightarrow$ closer packed arrangement	pressure, pore diameter, distance

3. deformation and fusion
   of curd grains $\longrightarrow$
   a. smaller curd surface area
   b. narrower pores
	deformability (hence: concentration,
	 temperature, pH, etc.), pressure, time

1+2 $\longrightarrow$ lower moisture content in cheese
3 $\longrightarrow$ higher moisture content in cheese

whey out

whey

FIGURE 22.11 Processes in a draining column of curd and whey. Highly schematic.

content of the cheeses. To overcome this problem the curd and whey mixture can be stirred gently in a buffer tank while being gradually decreased in temperature (see Fig. 25.9). In the meantime it can be transferred from the buffer tank to the filling/molding device.

The mechanization and scaling up of the curd forming that now have become normal practice has led to the use of a fixed time schedule for the various process steps. Adaptations during the manufacturing process, necessitated for instance by changing development of acidity or syneresis rate, are hard to achieve. One should therefore start from large quantities of milk (because this implies little variation in composition from one batch to the next) and precisely standardize the process conditions. Then, problems can still arise from varying rates of acid production caused by contamination by bacteriophages.

22.3   SHAPING AND PRESSING

In most cases, it is desirable to make the curd into a coherent mass that is easy to handle, is of a suitable size, and has a certain firmness and a fairly smooth closed surface. To achieve this, the curd is shaped; in hard and most semihard cheeses, this includes pressing of the curd.

Shaping of the curd can only be done if the grains deform and fuse (see also Fig. 22.11). *Deformation* is needed because the whole mass of curd must adopt the shape of the mold and because the grains should touch one another over nearly their total surface area. Viscous deformation is needed, i.e., the mass of curd must approximately retain its obtained shape when the external force is released. The greater the force, the faster the deformation; pressing thus can help. The deformation is considerably affected by the composition of the curd. With decreasing pH, the deformability increases until pH 5.2–5.3 is attained; at still lower pH, the curd becomes far less deformable. Furthermore, the deformability increases with the water content and especially with the temperature. At very high temperatures (e.g., 60°C) the curd can be kneaded into almost any shape and at a suitable pH it can even be stretched. This property is used in the manufacture of cheeses with ''pasta filata'' (Section 25.4). The high temperature and the kneading also affect the consistency, i.e., the cheese becomes tough and smooth. Poorly deformable curd (low pH, low water content, low temperature) can lead to holes in the cheese, even if it is heavily pressed. This may be the case for Cheddar cheese, but here it concerns fairly large pieces of curd, formed by cutting an already fused curd mass, and these pieces must undergo considerable deformation; moreover, the outside of these pieces of curd is firm due to the added salt. The applied pressure should therefore be high and the temperature not too low.

The *fusion* of the curd grains into a continuous mass is enhanced by increasing the area over which they touch one another. Obviously, conditions allowing easy deformation thereby enhance fusion. If, however, the curd still shows sig-

nificant syneresis during molding, fusion is counteracted by the layer of whey forming between the grains. The fusion proceeds most easily if the pH is fairly low, e.g., 5.5. This may be caused by new bonds forming readily between the paracasein micelles (the effect may be compared to the dependence of syneresis on pH; Fig. 22.7). Soured curd fuses poorly. If curd is stirred until its pH has dropped to 5.0 and is also cooled, it cannot be pressed into a cheese because the loaf immediately disintegrates.

Within a day after curd making, the fusion usually is complete, which means that no visible pores between curd grains are left in the mass of curd. The permeability coefficient has reduced to, say, 10^{-15} m^2. A few days may be needed to complete the fusion to the point where the mechanical properties of the cheese have become more or less homogeneous. Thus, if a piece of 1-day old cheese is deformed, it fractures between the original curd grains, whereas normally a cheese that is 4 days old fractures through the grains. These observations do not apply to cheese that is salted at the curd stage.

As mentioned above, the *pressing* furthers the shaping; it is needed (except for soft cheeses) to achieve a closed surface, i.e., to form a rind. It is not so much meant to decrease the water content. Moisture can be released from a mass of curd that is already more or less coherent because free whey or whey moving away from the curd grains can flow through the pores between the grains. If, however, a rind has formed, outflow of whey is markedly hindered and, accordingly, one of the effects of pressing is that any further decrease of the water content is small. The earlier the pressing starts, and the higher the applied pressure, the higher the remaining water content of the cheese. All of this applies to a not-too-dry and not-too-acidic curd. In such cases, the pressure usually ranges between 0.05 and 0.5 bar. In Cheddar cheese making, where the curd is much dryer and more acidic, pressures up to 2 bar are used, and vacuum pressing may be applied to prevent holes from remaining in the cheese.

Before and during pressing, a rapid temperature decrease must be avoided because it hinders the deformation of the curd grains and the rind formation. Larger loaves cool more slowly. (They may even rise in temperature due to the heat produced by the starter bacteria.) If the curd is not yet very dry, it can still show syneresis, and this proceeds more vigorously at a higher temperature; hence, the water content of the pressed cheese will be lower for a larger loaf size. Within an unsalted cheese mass, the water content is lower in the center than in the rind, with the difference amounting to, say, 6% water, as is illustrated in Figure 22.12. The above does not apply, however, to cheese that is made of curd stirred very dry. Applying a higher pressure to such curd results in a lower water content, as does a lower temperature. Presumably, the rind of this cheese only forms after a considerable proportion of the moisture has been squeezed out. In turn, the unevenness in the distribution of the water is much less.

Clearly, the *formation of a rind* is affected by the water content of the curd,

moisture content of cheese (%)
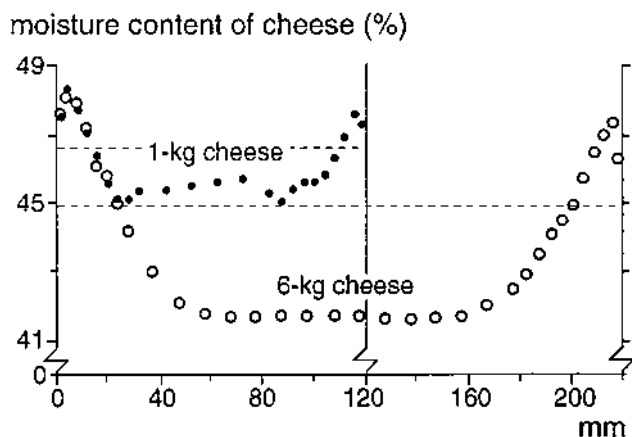
FIGURE 22.12 Distribution of the water throughout unsalted spherical loaves of cheese of 1 and 6 kg, respectively. The cheeses were shaped from one mass of curd, lightly pressed, and kept for several days. The broken lines indicate the average water content in a similar cheese of the same batch. From T.J. Geurts, *Neth. Milk Dairy J.* **32** (1978) 112–124.

the temperature and pressure applied during pressing, and the duration of pressing. A main factor is the local drainage of whey. If no drainage can occur, as in a mold of smooth nonperforated steel, the surface will not become closed. A closed surface does form, if moisture can be removed through perforations in the mold. Putting a cloth or a gauze between cheese and mold causes a still better drainage and results in a true rind, i.e., a thin layer of cheese, say, 1 mm thick, with a reduced water content, i.e., a kind of skin. The subsequent evaporation of water markedly enhances the thickness and toughness of the rind. Very fast evaporation may cause cracks.

22.4 SALTING

Salting is an essential step in the manufacturing of cheese. Salt is a substantial component of cheese with respect to preservation, flavor, consistency, usually the rate of ripening, often the rind, and sometimes shape retention.

The methods as applied in the salting of cheese can be classified as follows:

   a.   The salt is mixed with the ''dry'' milled curd pieces, e.g., Cheddar.
   b.   Dry salt can be rubbed onto the surface of the molded and pressed cheese, as was originally done in the manufacture of soft-type cheeses and of Edam. The rubbing is repeated several times.

c.   The cheese is kept immersed in a concentrated solution of NaCl (brine) until the desired amount of salt has been absorbed.

A combination of these methods may also be applied. For example, Gruyère cheese is brined, followed by rubbing dry salt onto the cheese surface. The way of salting affects the properties of the cheese. Salting has an effect on the cheese yield, i.e., salt penetrates into the cheese, while at the same time a greater amount of moisture moves out, resulting in a substantial loss of weight. The amount of salt-free dry matter of the cheese hardly decreases, e.g., by 2 g per kg cheese due to brining.

Dutch-type and several other cheeses are first molded and pressed. Then, after most of the lactose has been converted, brining takes place. For a relatively long time, the interior of the cheese remains without salt. Brining of cheese is a lengthy process; it often lasts for several days (see, for instance, Fig. 22.15). It requires much space but is easy to perform and does not often fail. A surplus of brine forms, however, leading to environmental problems. Direct mixing of dry salt crystals with milled curd is labor-intensive and less easy to control, partly because it implies considerable loss of salt with the ''press whey''; hence, it also causes an environmental problem.

The salt content of cheese can vary significantly among cheeses of different lots, but also among cheeses made of one batch of cheese milk. Many varieties of cheese contain, say, 2% salt (e.g., 4% to 5% if calculated on the water). The loss of weight during brining amounts to, say, 3%.

Some remarks on brine: pH, salt content, and temperature are important variables. In addition to NaCl, the brine has to contain sufficient calcium ions. If little or no ionic calcium is present, the cheese develops a velvety, soft, and easily damaged rind during brining, especially when weak brines are used. The defect must be due to peptization of the protein gel and is comparable to (local) swelling of the cheese. A surplus of brine is formed because the loss of moisture of the cheese exceeds the amount of salt absorbed. In actual practice, brine is never renewed but, of course, salt has to be regularly added.

22.4.1 Mass Transport During Salting

22.4.1.1 Transport of Salt

Figure 22.13 illustrates how the salt has penetrated into a cheese that has been brined for 8 days.

Salt penetrates into the cheese by diffusion. If the diffusion takes place through a plane surface (e.g., a cheese surface), it can be derived from Fick's law, i.e.,

$$\frac{C_b - C_x}{C_b - C_0} = \text{erf}(y) = \frac{2}{\sqrt{\pi}} \int_0^y e^{-\omega^2} d\omega \qquad (22.2)$$

g NaCl / 100g H₂O   water content (%)
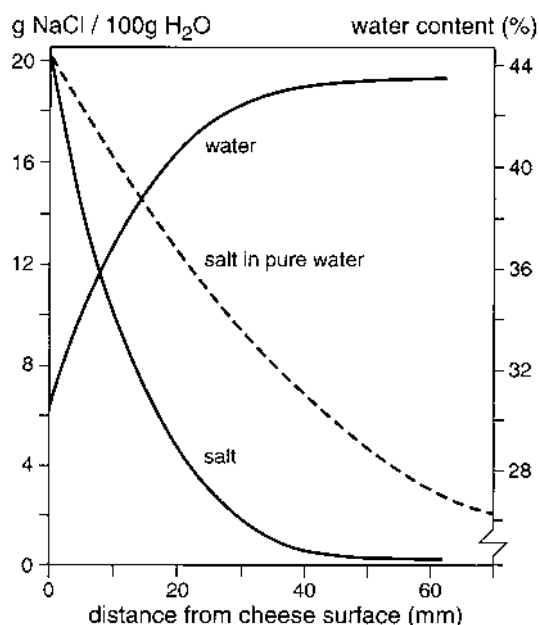
FIGURE 22.13   Distribution of salt and water in full-cream cheese after 8 days of salting in brine with 20.5 g NaCl/100 g H₂O. Also shown is what the salt distribution would be if the salt diffused in pure water.

where

$$y = x/(4D^* \, t)^{0.5} \tag{22.2a}$$

and where C is salt content relative to water in the brine (C_b), in the unsalted cheese (C_0), and in the cheese at a distance x from the brine/cheese interface (C_x); t is brining time and ω is an integration variable. From Eq. (22.2), the effective diffusion coefficient D^* (m² · s⁻¹) of the salt moving in the water in the cheese can be derived.

For example: $D^* \approx 2.3 \times 10^{-10}$ m² · s⁻¹ for a full-cream Gouda cheese with an initial water content of 45%. D^* is considerably lower than D, the diffusion coefficient of NaCl in water, which is approximately 12×10^{-10} m² · s⁻¹. In both cases, however, it concerns transport of salt in water as well as mutual diffusion because the boundary conditions and the concentration gradient for salt and water are equal, if opposite. The difference between D and D^* arises from the fact that in cheese the water is enclosed in a matrix, hence the salt moves in moisture

held in that matrix. Neither salt nor water can diffuse unhindered through the cheese. Consequently, $D^* < D$. The main factors responsible for impeding NaCl diffusion in cheese are:

    a. *Viscosity of the moisture*. The diffusion coefficient is inversely proportional to the viscosity of the medium available for transport. The viscosity of cheese moisture is greater than that of water, causing D^* to be as much as 10% lower than D.

    b. *Tortuosity effects*. The molecules (ions) diffusing in the water must travel by a circuitous route to bypass obstructing particles; hence the effective distance covered by the molecules will be longer. The molecules must bypass the fat globules; hence D^* decreases with increasing fat content. They must bypass the particles of the protein matrix in the fat-free cheese; hence D^* decreases with decreasing water content in the fat-free cheese.

    c. *Mechanical sieve action*. The pore width of the protein matrix in cheese is some 1–3 nm, depending on the water content. The diameter of the hydrated salt ions is at least 0.5 nm. Hence, a marked frictional effect is exerted on the diffusing ions.

    d. *Counterflux*. During the salting process, salt penetrates into the cheese and at the same time much water moves out. The water flux considerably exceeds the salt flux; hence the cheese shrinks (in the region into which salt has penetrated). The counterflux of water reduces the apparent rate of the salt diffusion.

The effective diffusion coefficient D^* greatly depends on the initial water content (see Fig. 22.14). For example, in a full-cream cheese with 50% water D^* is more than twice the value in a cheese with 39% water. Moreover, for one and the same water content, D^* generally increases with the fat content; this is because a higher fat content goes along with a higher water content in the fat-free cheese, and the latter factor has a stronger effect on D^* than the fat content has.

22.4.1.2 Displacement of Water

Where salt penetrates, water diffuses out of the cheese (see Fig. 22.13; the initial water content of the cheese was approximately 43%). The salt and water transports are quantitatively related. The proportionality factor or flux ratio p is defined as the ratio between the decrease in water content and the increase in salt content at distance x from the cheese–brine interface and at time t. p may be fairly constant within a cheese, i.e., independent of x or t. During the process, the water content thus is lowest in the part of the cheese closest to the brine, whereas it remains unchanged where salt has not yet penetrated.

    The flux ratio p approximates 2.5. The migration rate of the water surpasses
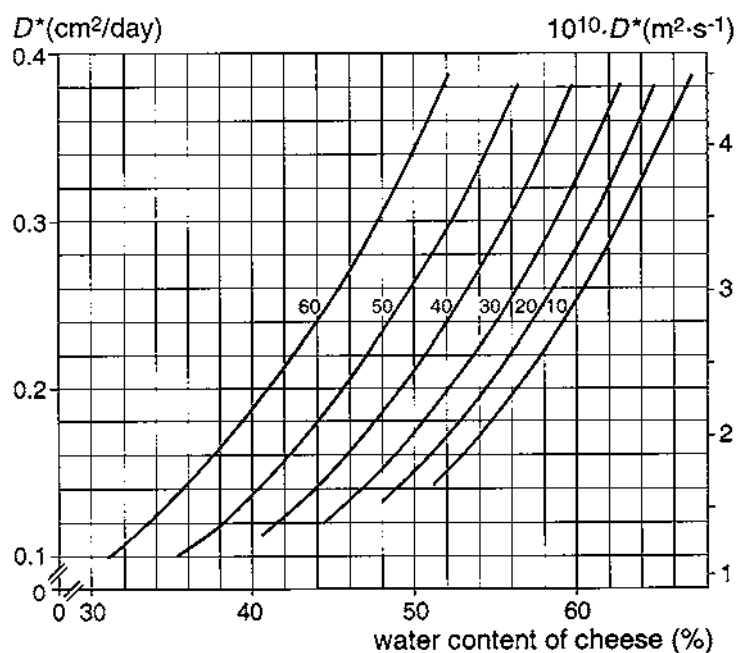
FIGURE 22.14 Diffusion coefficient of NaCl in the water in cheese (D^*) as a function of the initial water content of the cheese. Parameter is g fat/100 g dry matter in unsalted cheese.

the rate of the salt at every moment and at every location in the cheese. This is largely due to partial osmosis because the hindrance of H_2O diffusion in cheese by the narrowness of the pores in the cheese matrix is less than that of NaCl diffusion. The outward migration of the water thus is a direct consequence of the penetration of the salt. The water is not displaced as a result of an independent shrinking of the matrix, but the reduction of cheese volume follows from osmosis. The cheese matrix can fully or partly comply with the forces causing this reduction; if the cheese is fairly rigid (low pH, low water content, low temperature), the matrix cannot fully comply, and less water is lost than for a less rigid cheese matrix, i.e., p is smaller.

The extent to which the average water content of the salted part of the cheese during salting is lower than the initial water content depends on several factors, but the length of the brining time does not affect it. Data are given in Tables 22.1 and 22.2.

22.4.1.3 Quantity of Salt Taken Up

The salt concentration of the cheese moisture as a function of x and t can be calculated by using Eq. (22.2). The effective diffusion coefficient D^* is independent of time t (Table 22.2).

Provided that the salt has not yet penetrated into the center of the cheese, the quantity of salt absorbed from a flat cheese surface follows from:

$$M_t = 2 (C_b - C_0) (D^*t/\pi)^{0.5}\, \bar{w} \tag{22.3}$$

where M_t = quantity of salt absorbed over time, in kg NaCl/m^2; C_b = kg NaCl/m^3 water in brine; C_0 = kg NaCl/m^3 water in unsalted cheese; $\bar{w}$ = average water content, expressed as a fraction of the cheese (kg/kg). For $\bar{w}$, the weighted average of the water content in that part of the cheese in which the salt penetrates should be taken; the weighting factor is the local salt uptake and Table 22.1 gives the approximate difference Δ with the initial water content. Inserting the original water content of the cheese in Eq. (22.3) would result in the calculated salt uptake being too high: $\bar{w}$ is much lower than the original water content due to water moving out during the salting process.

Obviously, the quantity of salt absorbed is not proportional to t, but to $t^{0.5}$. Soon, M_t increases even more slowly than would fit in with Eq. (22.3) because of the limited dimensions of the cheese. The greatest limitation of salt absorption

TABLE 22.1 The Difference (Δ) Between the Initial Water Content and the Weighted Average Water Content of Cheese, and the Mass Flux Ratio (p) of Water to Salt

Fat in dry matter (g/100 g)	Initial water (g/100 g)	pH of cheese	Temperature (°C)	Salt in brine (g NaCl/ 100 g H$_2$O)	Δ (g/100 g)	p (g/g)
10	59	5.0	12.5	20	10	2.7
40	49	5.0	12.5	20	8.5	2.5
60	39	5.0	12.5	20	6	2.3
50	36	5.0	12.5	20	5	2.5
50	45	5.0	12.5	20	7.5	2.4
50	50	5.0	12.5	20	8.5	2.2
50	45	4.7	12.5	20	6	1.7
50	45	5.7	12.5	20	8.5	2.8
50	45	5.0	20	20	10	2.8
50	45	5.0	12.5	14	4	1.8
50	45	5.0	12.5	31	11	3.2

occurs where the surface is markedly curved, i.e., near edges. The smaller the cheese loaf (the more the surface is curved) and the higher the salt content of the cheese, the stronger this effect.

The mass fraction of salt (Z) in the brine-salted cheese can be derived from:

$$Z = M_t A / G_s \qquad (22.4)$$

where A and G_s are surface area (m^2) and weight (kg) of the cheese, respectively. Textbooks for process engineering deal with diffusion in bodies of various geometry, needed for adjustment of Eq. (22.3). On the basis of the appropriate equations, the process variables to arrive at a desired salt content in the cheese can be calculated (including brine strength and length of brining time).

22.4.1.4 Water Content of the Brine-Salted Cheese

On the basis of the flux ratio p (Table 22.1), the water content of the cheese after the salting (w_s, kg/kg) can be predicted [see Eq. (22.5)], as well as the weight loss. During brining, S kg salt per kg cheese solids-not-salt is absorbed and pS kg water is lost. Accordingly, after the process the cheese contains $H - pS$ kg water per kg solids-not-salt, H being kg water per kg solids-not-salt before the salting of the cheese. Then

$$w_s = \frac{H - pS}{H - pS + S + 1} \qquad (22.5)$$

22.4.1.5 Loss of Weight of Cheese

The loss of weight of cheese during salting approximates $pm - m$, where $m =$ quantity of salt taken up (pm is the loss of water). On average, the weight loss equals about 1.5 m. Expressed as a fraction of the weight of the cheese before salting (G_i), the weight loss (V) follows from

$$V = m(p - 1)/G_i \qquad (22.6)$$

The above applies to brine salting of cheese. By and large, it is also true of *dry salting* of cheese. If the curd is salted before pressing, the same processes occur but the quantitative relationships differ considerably and are hard to predict and control. In most cases, as in Cheddar cheese manufacture, the curd first sediments and fuses to a compact mass, which is subsequently cut into strips. Then dry salt is mixed with the curd. Salt diffuses inward, causing a counterflow of whey from the curd to the surface, which creates a brine solution around the curd strips. After approximately 10 min the absorption of the appropriate amount of salt has been achieved, but in addition to the curd a fair amount of salt solution is present, which is discarded during subsequent pressing. In this way, up to 50% of the added salt may be lost, especially if the salt is inadequately mixed with

the curd. An increase in salting rate increases the relative salt losses with the press whey, and it is very difficult to obtain high salt concentrations in the cheese. A more precise dosage is possible if the curd grains are not allowed to fuse before the addition of salt, as in the manufacture of cottage cheese and some Cheddar-type cheeses.

22.4.2 Important Variables

Factors affecting D^*, p, salt content, and water content of the cheese after brining are given in Table 22.2. Figure 22.15 gives results as a function of brining time.

D^* is primarily affected by water and fat content (Fig. 22.14). The temperature also has a limited effect. Within the range pH 4.7–5.7, D^* does not significantly depend on the pH.

Flux ratio p is scarcely affected by the water content, but all other factors have an effect (Tables 22.1 and 22.2).

Salt and water content after the brining process greatly depend on initial water content, fat content, the ratio of surface to mass, salt content of the brine,

TABLE 22.2 Influence of Several Important Factors on the Salting of Cheese[a]

Factor	Weighted average water content	Quantity of water lost	D^*	Quantity of salt taken up	p
			Effect on:		
Fat content	−	−	−	−	−
Water content	+	+	++	++	±/−
pH of cheese	−	+	0	−	++
Ratio of surface to weight	0	++	0	++	+
Temperature	−	+	+	+	+
Duration of brining	0	++	0	++	++/+/0/−
Salt content of brine	−	++	0/−[b]	++	++
pH of brine	+	−	?	+	−

+, positive correlation; ++, strong positive correlation; ±, correlation questionable, but at most slight; 0, no correlation; −, negative correlation; ?, not investigated.
[a] Weighted average water content refers to that part of the cheese in which the salt has penetrated. D^* is effective diffusion coefficient of the salt in the water in the cheese. p is mass flux ratio water/salt. Quantities mentioned are per kg of cheese.
[b] D^* may be slightly lower if the salt concentration of the brine is very high.
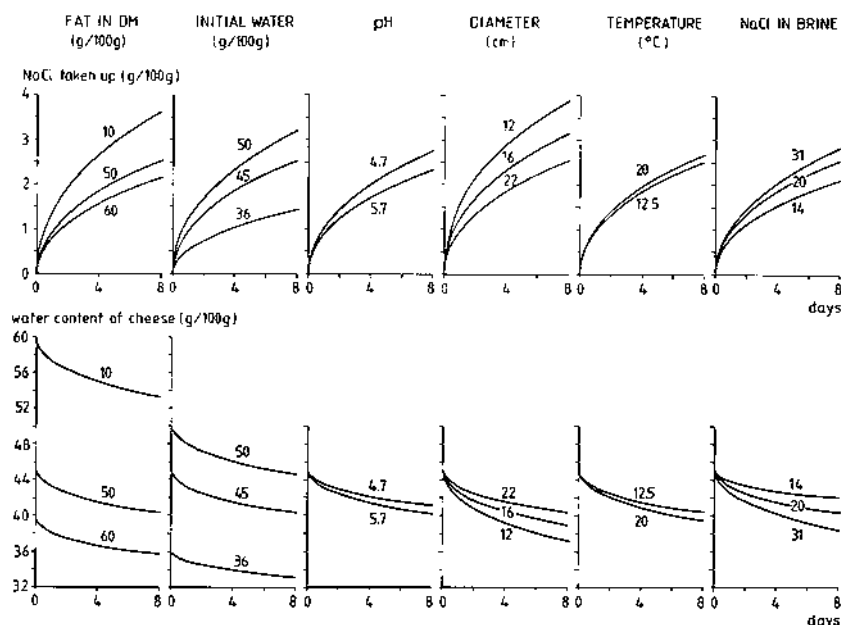
FIGURE 22.15 Predicted effects of some factors on the salt uptake and on the water content during salting of a spherical cheese with 50% fat in the dry matter, 62% water in the unsalted fat-free cheese (hence, a full-cream cheese contains 45% water), pH 5.0, diameter 22 cm (~6 kg), in brine of pH 5.0 containing 20 g NaCl/100 g water, temperature 12.5°C (unless stated otherwise).

and, of course, the duration of brining. The temperature and pH of cheese and brine have less effect.

It is important to note that nowadays many cheeses are pressed for a relatively short time and are brined fairly soon afterward; see, e.g., Figure 25.9. Such conditions more or less deviate from those mentioned in this section. Uptake of salt by processes other than diffusion then is possible, and the total amount taken up generally is somewhat higher than predicted.

22.4.3 Distribution of Salt and Water After Salting

After the salting, water and salt become more or less evenly distributed throughout the cheese mass. For Edam cheese it takes some 4–6 weeks, for 4-kg Gouda cheese 8 weeks, for Camembert and Brie 7–10 days, for Emmentaler over 4 months. A completely even distribution of the salt is rarely if ever reached, if only because of ongoing evaporation of water.

If the cheese is salted at the curd stage, the salt initially is fairly unevenly distributed, especially when the curd is cut to relatively large strips, as in Cheddar cheese manufacture. It then takes a long time to arrive at an even distribution of the salt. As an example, after a week the relative standard deviation between samples taken from one cheese was found to be 25%, whereas after 9 weeks it was still 10%. This must be ascribed to inadequate mixing of salt and curd because after such a long time the differences between the salt contents at various spots in one strip of curd (diameter about 2 cm) should have disappeared.

22.4.4 Diffusion Rate of Other Components

The theory of transport of salt in cheese also applies for diffusion of many other components. Increasing the size of the molecules results in a considerable decrease of the effective diffusion rate in the water in cheese. Protein molecules (e.g., enzymes) diffuse very slowly. For instance, $D*$ of chymosin is approximately 3.5×10^{-12} m$^2 \cdot$ s^{-1}. The diffusion rate of low-molar mass compounds like lactic acid is comparable to that of sodium chloride. Some compounds, e.g., CO_2, also dissolve in the fat. In these instances prediction of the diffusion rate becomes more difficult.

22.5 STORAGE AND HANDLING

In this section the basic principles of the storage of cheese are described. Types of cheese that are cured are considered, i.e., the types produced most (Section 25.2 deals with nonripened, fresh cheese). We can distinguish:

    a. Types of cheese with a specific surface flora or internal molds, in addition to the normal flora of lactic acid bacteria
    b. Types without a specific flora

The storage of cheese starts after its manufacture. Often this is after the salting. In Cheddar cheese making salt is mixed with the curd before pressing. Feta-type cheeses are first salted, after which they are packed and cured in brine or in acid whey with salt added. This holds also for the Domiati type of cheese; in its manufacture the milk is provided with a high salt content (8% to 15% NaCl), whereas acid is produced in the cheese by salt-tolerant lactic acid bacteria.

The storage of maturing cheese is aimed at making and keeping it suitable for consumption. The product should develop the properties that are characteristic of that type of cheese: flavor, consistency, body (cross-sectional appearance), rind. Any loss, especially that caused by excessive evaporation of water, as well as deterioration of the rind and/or of the texture due to undesirable microbes and insects (cheese mite) should be prevented.

For some cheeses, the handling during curing requires more labor than the manufacture proper.

The actual treatment significantly depends on the type of cheese involved and varies with the progress of maturation. Various types have a short ripening time and shelf life, whereas other cheeses are adapted for extended storage (Table 22.3). The following sections cover the main variables.

22.5.1 Temperature

Temperature affects the growth rate of microbes of a desirable specific flora and the activity of their enzymes as well as that of enzymes of foreign origin, especially rennet and starter; it thereby affects the rate of ripening. Generally, a higher temperature causes a quicker ripening, but at the same time it enhances the risk

TABLE 22.3 Approximate Examples of Storage Conditions During Cheese Ripening and Storage and of Ripening Time of a Number of Cheese Types

Type of cheese	Temperature (°C)	Relative air humidity (%)	Ripening time (days)
Soft cheese without surface flora (e.g., Meshanger)	12–14	95	15–20
Soft cheese with red smear (e.g., Muenster)	12–16	Often >95	35
White molded cheese (e.g., Camembert)	(1) 10 d 11–14 (2) 4 (packed)	85–90	35
Blue-veined cheese (e.g., Roquefort)	7–10	95	100
Gouda and Edam types	12–16	85–90	50–300
Semihard cheese with surface flora (e.g., Tilsiter)	12–16	90–95	150
Hard cheese with surface flora (e.g., Gruyère)	(1) 2 weeks 10–14 (2) 5–10 weeks 16 (3) remainder 10–14	High 85–90 85	300
Emmentaler	(1) 2 weeks 10–14 (2) 5–10 weeks 20–24 (3) remainder 10–14	80–85 80–85 85	100–200
Cheddar types	(1) 2 weeks 12–16 (2) remainder 5–7	75–80	e.g., 150
Parmesan	(1) 1 year 16–18 (2) 1 year 10–12	80–85 85–90	700

of spoilage by undesirable microbial growth. Examples are undesired mold growth on the surface and butyric acid fermentation. Types of cheese that are susceptible to the latter fermentation may be cured at low temperature during the first ripening stage, to allow the salt to become evenly distributed throughout the cheese (see Section 24.2). This way of working is especially applied for cheese intended for the making of processed cheese.

If the temperature is too low, the rate of ripening is unsatisfactory. At very low temperature the flavor remains flat and uncharacteristic. Storage at low temperature normally serves to slow down continuing ripening processes, after a preceding ripening time at a higher temperature, and to retard approaching defects. It thus extends the storage time. In particular, soft and prepacked cheeses are treated in this way. Various types are also suitable for freezing, especially if packed in small containers. For example, Gouda and Cheddar cheeses can be stored for more than 6 months at −3°C. If stored at −20°C these cheeses turn mealy or even crumbly. A temperature of −20°C is suited to store high-fat Gouda cheese, with 60% fat in the dry matter.

The temperature also affects the evaporation of water from the cheese.

22.5.2 Air Humidity and Air Velocity

Air humidity and air velocity affect the evaporation of water. The air humidity has a considerable effect on growth of desirable and undesirable microorganisms on the cheese rind. To allow the cheese to retain a satisfactory shape, the ripening cheese loaves should be turned, initially frequently. Such turning should also enhance the growth of any aerobic flora on the whole cheese surface and prevent, in cheese without a specific surface flora, the growth of microaerophilic microorganisms between loaf and shelf. Incidentally, the air humidity in the vicinity of the cheese surface (the ''microclimate'') can appreciably differ from that elsewhere in the storage room.

Rate and extent of evaporation can be partly responsible for developing microbial defects in that the cheese does not become dry enough. On the other hand, the cheese should not dry too quickly, especially not just after brining, as this may cause cracks in the rind. (Sometimes the cheese loaf is rinsed with water after brining, causing the rind to become more supple.) Initially, the relative humidity may be taken somewhat lower and the air velocity higher, if the cheese is not pressed or if it is pressed in such a way as to form a weak rind; evaporation causes the rind to become firmer. If much water evaporates the cheese rind turns into a closed horny layer that slightly retards the transport of water and gases. Of course, evaporation implies loss of weight. This loss approximates 0.2% per day for the first 2 weeks in Gouda cheese (10-kg loaf). In Table 22.4 examples are given of the weight loss of cheese stored under various conditions.

TABLE 22.4 Loss of Weight of Gouda Cheese Kept for 9 Days Under Various Conditions

Air velocity (m/s)	Temp. (°C)	Relative humidity (%)	Original weight of cheese (kg)	Loss of weight (%)
0.1	14	85	10	1.7
0.2	14	85	10	2.2
0.4	14	85	10	2.6
1	14	85	10	3.2
0.2	12	85	10	2.1
0.2	16	85	10	2.3
0.2	14	82	10	2.6
0.2	14	86	10	2.1
0.2	14	90	10	1.4
0.2	14	85	4	2.7
0.2	14	85	15	2.1

After S. Bouman, *Zuivelzicht* **69** (1977) 1130–1133.

22.5.3 Rind Treatment

22.5.3.1 Cheese with a Specific Flora

The reader is referred to Section 25.6. In this category we distinguish:

1. *Cheese with a red smear*. Coryneforms (e.g., *Brevibacterium linens*) are essential. These do not grow if the pH in the cheese surface is too low. Lactic acid should first be decomposed, which is mainly effected by yeasts. A supply of oxygen (fresh air) stimulates the growth of the bacteria. Regular smearing of the surface or washing the cheese with water or with weak brine aids in developing a uniform slimy layer. The necessary bacteria disappear if the cheese is washed too frequently or too intensely. The slimy layer inhibits mold growth. There are numerous types of soft cheese with a read smear, e.g., Muenster, Limburger, Pont l'Évêque, which are all of small size. Examples of semihard cheeses with a smear on the rind are Tilsiter, Port Salut, and Kernhem cheese. An example of a hard cheese is Gruyère. As time passes the slimy layer is generally left to dry. Afterward, certain cheese types are coated with latex.

2. *White-molded cheese*. The cheese may be sprinkled with a mold culture after it has been salted and partly dried, or mold spores can be added to the cheese milk and/or the brine. Growth conditions can be enhanced by adjusting the temperature in the ripening room and by a high relative

humidity (which should, however, be lower than that for the preceding group of cheeses). Furthermore, the development of a white mold is stimulated by keeping the cheese in the dark. During maturation, contamination of the cheese surface by undesirable molds should be prevented.

3. *Blue cheese*. Before the ripening starts, the cheese is perforated with needles. Cylindrical loaves are put down on their round sides to stimulate the air supply into the pores formed, which enhances the growth of the mold added to the curd. The cheese is cured at relatively low temperature and at high relative humidity. Most of the blue-veined cheeses should not develop a significant surface flora and are thus to be kept clean. Other types, like Gorgonzola, do have such a flora.

22.5.3.2   Cheese Without a Specific Flora

Here we distinguish:

a. *Hard and semihard, brine-salted cheese*. Microbial growth on the rind of the cheese may adversely affect cheese quality, especially flavor and appearance. Of particular importance is the growth of molds (some of which may produce mycotoxins), coryneforms, and yeasts. To avoid such growth, the cheese rind is supplied with a surface coating. Currently, a latex or plastic emulsion is generally applied, i.e., a polymer latex of vinyl acetate, vinyl propionate, or dibutyl maleinate. On drying, a coherent plastic film forms that slows down evaporation of water and offers a better protection against mechanical damage than did earlier used expedients like linseed oil and paraffin oil. The latex coating allows the cheese rind to be much weaker. The mechanization and speeding up of the manufacture of many types of cheese would not have been possible without the introduction of these latex emulsions. The film mechanically hinders mold growth, be it incompletely. It may also contain fungicides, e.g., natamycin (= pimaricin), an antibiotic produced by *Streptomyces natalensis*, or calcium or sodium sorbate. In almost all European countries only natamycin is permitted. When compared to sorbates, it offers the advantages that its protective action is about 200 times as strong, that its migration into the cheese is limited to the outer few millimeters, and that it does not negatively affect the appearance, taste, and flavor of the cheese. Moreover, it is harmless. An acceptable daily intake of 0.3 mg natamycin per kg body weight has been proposed (note that the outermost cheese rind is rarely eaten). The amount applied to cheese rind is generally below 2 mg/dm^2.

   In practice, successive treatments with latex emulsion are applied

to all sides of the cheese shortly after brining. During long curing the treatment may be repeated. The surface should be sufficiently dry before each treatment. The conditions in the ripening room must permit the emulsion to dry quickly (not too quickly because cracks could form in the film, leading to mold growth in the cheese). Too slow a drying may cause growth of microorganisms, especially coryneforms and yeasts. Such growth is greatly enhanced by the high humidity between loaf and shelf, caused by moisture that is inevitably expelled by the cheese at the beginning of the ripening. To prevent this, as well as to allow the cheese to retain a satisfactory shape, the loaves are frequently turned during this stage; the frequency is reduced upon prolonged curing. Obviously, regular cleaning and drying of shelves should form part of a general program on hygiene in curing rooms.

b. *Cheese salted at the curd stage*. Formerly, Cheddar cheese and related rather dry cheeses were often stored in cheese cloth and only provisionally kept clean. Currently, the cheese is usually treated with paraffin wax shortly after pressing and packed in cardboard or wooden containers, requiring little if any further attention. At first, the loaves should be piled not too close together in order to allow cooling. If the cheese still shows some syneresis (high water content, high temperature), an aqueous layer forms between the cheese rind and the paraffin coating in which deteriorative microorganisms can proliferate.

22.5.4 Packing

Packing is an important aspect of the objective of storage of ripening cheese. Several factors are involved in selecting a package: type of cheese and the consequent resistance to mechanical damage (hard or soft cheese); presence of a specific flora; wholesale or retail packaging; permeability to water vapor, oxygen, CO_2, NH_3, and light; labeling facilities; migration of flavors from package to product; the system for storage, distribution, and sale (supermarket, specialist shop, rate of turnover in the market). These aspects cannot be discussed in detail here but a few remarks will be made.

a. Formerly, semihard cheese was often treated with paraffin wax, whereas currently many are coated with a latex emulsion which, of course, is also a kind of packing. When the cheese is going to be waxed its surface should be very clean and dry; otherwise growth of bacteria between cheese rind and paraffin wax coating will cause problems, especially because of gas production and off-flavors. Waxing thus can be applied for low-moisture cheese shortly after manufacture, whereas cheese with a higher water content may be waxed only after the required rind properties have been attained.

b. Some cheese is cured while being packed in an air and water vapor–tight shrinking film, e.g., Saran wrap. The cheese may be made in rectangular blocks, which are usually designed for sale in prepacked portions or slices, or for the processed cheese industry. Compared to normally ripened cheese, important differences are as follows:

1. The cheese has no firm rind.
2. Its composition is more homogeneous due to moisture losses being quite small.
3. The cheese has a lower water content immediately after manufacture, since this content must meet the requirements for a ''normal'' cheese after ripening (which loses more water during storage).
4. The starter may not produce too much CO_2, since otherwise loosening of the wrapping would readily occur (''ballooning'').
5. Often the curing temperature is taken lower. Together with differences in composition, this causes the flavor development to be less than in normally ripened cheese of the same age.
6. The cooled blocks can be piled up closely and need not be turned.

22.6 STANDARDIZATION AND YIELD

Standardization of milk for cheese making means adjustment of its fat content to ensure that the cheese being made contains the legally required percentage of fat in the dry matter (FDM). The yield is the mass of cheese obtained from a certain quantity of the standardized milk.

It may be of economical importance to precisely calculate the desired fat content of the cheese milk and every producer wants to predict the cheese yield. Comparison with analytical results may enhance our understanding of the cheese-making process. All of the cheese made from one vat of milk may be weighed. If this is always done in the same way it may be a valuable help because it gives a first indication of the composition of the cheese.

22.6.1 Standardization

Under practical cheese-making conditions, establishing the correct fat content of the cheese milk causes specific problems. First, a cheese loaf is always inhomogeneous in composition, especially in brine-salted and in all small cheese loaves, causing problems in establishing the real fat-in-dry-matter content. For this reason, borer samples may give considerable bias and the whole loaf, a sector from it, or a quarter from a square-shaped cheese is to be taken, ground, and thoroughly mixed. Second, the fat contents of different loaves from one batch are not identical, the standard deviation often amounting to about 0.5% FDM. Moreover, FDM decreases during ripening, since proteolysis involves conversion of some water

to dry matter. For these reasons, a safety margin is taken into account, i.e., the initial fat content is adjusted to a somewhat higher level than is required. A surplus value of, say, 1.5% FDM is often taken. (The plus sign in notations like 40+ or 60+ refers to this margin.)

Difficulty of standardization is enhanced by the multiplicity of variables affecting the ratio of fat to dry matter in the finished product. These include the following:

a. *The composition of the milk*. This changes with the season and shows short-term fluctuations. Moreover, changes can occur during prolonged cold storage. Previously, the fat content of the whole milk was taken as the basis of standardization, based on an assumed constant fat-to-protein ratio. The higher the fat content of the whole milk, the higher the fat content of the cheese milk has to be. The ratio of the fat to the protein content of the whole milk is, however, not constant. Hence, this method is not very precise. Greater certainty is obtained if the protein content of the milk or, still better, its casein content is estimated. Almost fixed proportions of the fat and of the casein micelles are carried over from the milk into the cheese, provided that the manufacturing process is kept constant.

b. *The method of making the cheese*. Important aspects are:

   1. The intensity of pasteurization of the milk; denatured serum proteins are incorporated in the curd, increasing its SNF content.
   2. The manner of cutting the curd, which affects fat losses into the whey.
   3. The quantity of wash water used, which affects the SNF content of the cheese.
   4. The amount of acid produced in the curd and thereby the loss of calcium phosphate into the whey.
   5. The quantity of salt taken up by the cheese.

c. *The maturation of the cheese*. The quantity of fat hardly changes but the SNF does, since water is converted to dry matter due to hydrolytic processes.

To standardize the cheese milk, the ratio of its fat content (f) to its crude protein content (p) may simply be used as a basis. If F is the fraction of the fat that is transferred from the milk to the cheese, and if K kg fat-free dry cheese, including added salt, is formed from 1 kg of milk protein, then Ff/Kp represents the ratio of fat to SNF in the cheese. As far as the manufacture of Dutch-type cheese is concerned, both F and K approximate 0.9. Hence, the ratio of fat to protein in the milk may be adjusted to the ratio that is desired between fat and SNF in the

cheese. Schulz and Kay accordingly arranged some tables: if p is known, the appropriate value of f may be found in the table for any type of cheese.

More elaborate formulas may be used, e.g.,

$$f = rp + q \qquad\qquad (22.7)$$

Under usual conditions of Gouda and Edam cheese making, r primarily depends on the desired fat-in-dry-matter content of the cheese. For 40% FDM, $r \approx 0.67$; for 48% FDM, $r \approx 0.91$ (see also Fig. 22.16). The parameter q refers to the fat lost into the whey. The loss increases more than proportionally with f. For cheese with 20% FDM, $q \approx 0.05$; for 40% FDM, $q \approx 0.14$; for 48% FDM, $q \approx 0.20$; for 60% FDM, $q \approx 0.40$. In Figure 22.16, f is shown as a function of FDM; note the strong increase of f with increasing FDM.

22.6.2   Yield

The reader is referred to Figure 21.1. The yield of cheese can be defined as kg of product (y) obtained from 100 kg of cheese milk:

$$y = \text{kg (fat + protein + other solids + water)} \qquad\qquad (22.8)$$
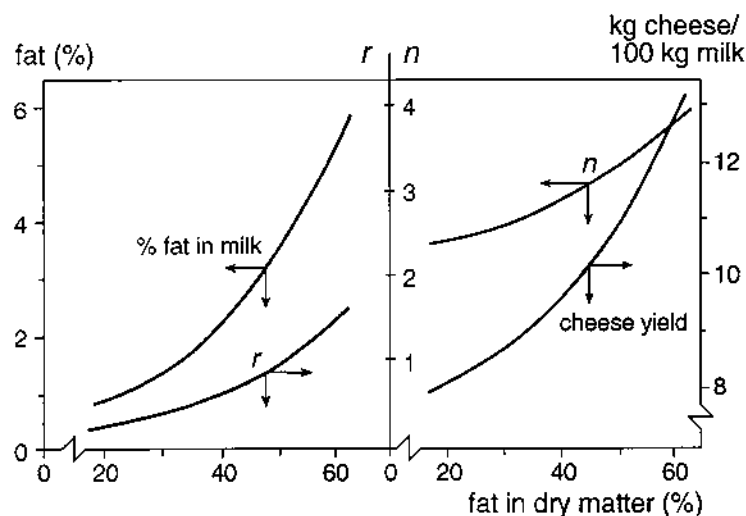
FIGURE 22.16   Factors r [Eq. (22.7)] and n [Eq. (22.9)]. Examples of the fat content of the cheese milk (3.4% protein) and of the yield of cheese (12 days old, 58% water in the fat-free part, 4% salt in dry matter) as a function of the fat-in-dry-matter content.

Most factors affecting the ratio of fat to dry matter also affect cheese yield. An important variable is the water content of the cheese and thereby the loss of whey (syneresis) during curd making and pressing. Many factors affect the water content (Section 22.2.2). For Dutch-type cheese, its standard deviation between loaves of one batch often amounts to 0.5% to 1%. An increase of the water content by 1 percentage unit increases *y* by, say, 0.2 kg.

  If we consider the water content as given, additional factors affecting *y* are for the most part via the casein content of the milk:

  a. *Season*. In western Europe, the yield may be relatively high in autumn and low in spring, the discrepancy amounting to over 10%.
  b. *Mastitis*. Severe mastitis leads to the production of milk with a reduced casein content and a lower ratio of casein to total N. Actually, since large quantities of bulk milk are used, real problems are seldomly met.
  c. *Genetic variants of milk proteins*. These may affect cheese yield, presumably because milk composition is somehow correlated with specific genetic variants, especially those of β-lactoglobulin.
  d. *Cold storage of the milk*. The extent of a possible effect is not quite clear. Unequivocal results are difficult to obtain from experiments, since purely physicochemical changes must be separated from bacterial effects. It is, however, clear that proteolysis generally causes a loss of yield.
  e. *Pasteurization of the milk*. A higher heating temperature will increase *y* (Section 22.1.2).
  f. *Rennet type*. Differences in proteolysis, other than the splitting of κ-casein, would affect *y* but usually they are negligible, unless some microbial rennets are used.
  g. *Starter*. A change in the percentage added introduces several other changes. First, the incorporation of denatured serum proteins into the curd increases with the quantity of starter (which is made from intensely heated milk) and thereby in itself increases the yield. Second, for several types of cheese the use of more starter inevitably requires more curd-washing water (Section 22.2), which increasingly dilutes the whey and, hence, reduces the yield (see below). The net result of both factors may be almost nil. Increasing the rate of acid production, by adding more or more active starter, decreases the pH of milk and curd, inducing more dissolution of Ca and inorganic phosphate (Fig. 22.17; see also Fig. 22.9). Probably, the subsequent loss of yield is fairly small, say 30 g for 10 kg cheese produced if the pH at separation of curd and whey is as low as 6.25 instead of 6.5 (see also Fig. 25.10). Moreover, more proteose-peptone will leave the micelles at a lower pH and will be lost into the whey. Accordingly, a lower yield, i.e., a

g / 100g
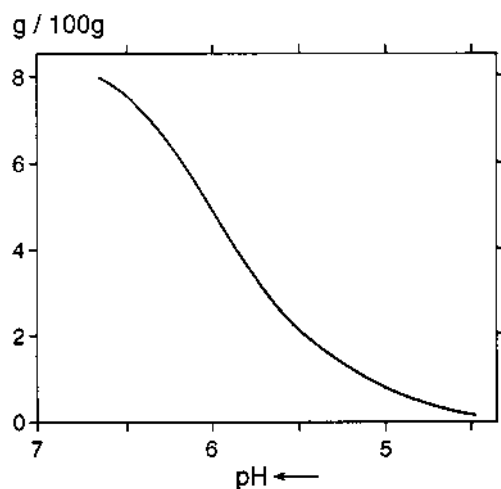
FIGURE 22.17 Quantity of mineral components associated with the casein micelles (the "colloidal calcium phosphate") in g per 100 g paracasein as a function of pH. Approximate average values.

higher protein content of the whey, might be expected, but experimental evidence seems to be lacking.

h. *CaCl₂*. Addition to the milk causes some accumulation of colloidal calcium phosphate in the micelles. For several types of cheese about 1 mmol CaCl₂ per liter is added to enhance the clotting, presumably enhancing yield by some 30 g per 100 kg milk (i.e., about 0.3%).

i. *Washing*. The dilution of whey with water affects *y*. Increasing the quantity of added water, e.g., from 30% to 40% of the mass of curd and whey after part of the whey has been let off, reduces *y* by 0.5% to 1%. The effect is illustrated in Figure 22.18. In calculating *y*, the efficiency of decreasing the concentration in the curd particles by the washing was taken to be 90% for lactose and other low-molar mass substances, and 50% for the serum proteins.

j. *Ultrafiltration*. An increase in cheese yield may be obtained because of the accumulation of serum proteins in the curd. Using a concentration factor lower than 3 hardly increases the amount of serum proteins in the cheese, but partial concentration to a higher factor does. If no whey is released after ultrafiltration of the milk, all serum proteins are retained in the cheese (see also Section 22.2.1).

k. *Salting*. Absorption of NaCl obviously causes a gain in weight of the
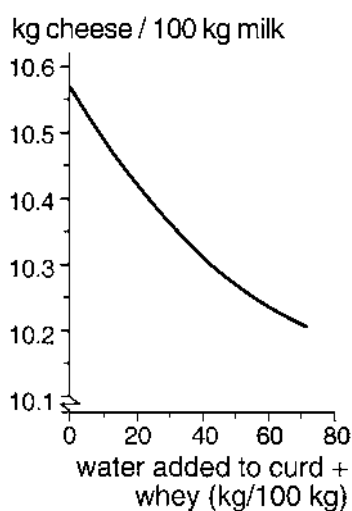
FIGURE 22.18 Example of the yield of Gouda cheese (12 days old, 41% water) as a function of the quantity of curd washing water used. Water content and pH of the cheese are assumed constant. Adapted from Posthumus et al.

cheese. If the cheese is brined, however, there is a greater loss of moisture, hence a net loss of weight. The quantity of salt absorbed varies, e.g., from 1% to 3%, and the net weight loss may vary from, say, 2% to 6% (0.02 to 0.06 kg per kg of cheese produced). The loss of solids-not-salt, including losses caused by mechanical damage during salting, ranges from 1 to 3 g per kg. Also in the case of dry salting, as for cheddar types, considerable moisture loss occurs, e.g., 0.04 kg per kg cheese.

1. *Curd making.* Due to cutting and stirring 5% to 10% of the fat is lost in the whey. Also, curd fines are lost (0.2% to 1% of the protein).

The following refers specifically to Gouda-type cheese. Strictly speaking, *y* refers to the ultimate product, excluding curd remnants that are lost (about 10 g per kg of cheese produced, of which 5% to 10% is curd fines); *y* includes any latex coating (<5 g/kg). Eventually, evaporation causes some loss, e.g., 10 g/ kg for a 10-day-old cheese. Clearly, *y* can in no way be predicted very precisely, the cheese making process being too complex. Even the random variation in water content (see above) makes exact prediction difficult. Prediction of the yield of cheese from a given vat of standardized milk will obviously be based on its

protein content. In the mentioned tables of Schulz and Kay, the y value can be looked up if cheese of certain fat and water content and with 5% salt-in-moisture has to be made, starting with milk of known protein content. The yield can also be calculated from the formula (for 12-day-old Gouda cheese):

$$y = np - 0.084 \tag{22.9}$$

where n depends mainly on FDM. In the case of full-cream cheese ($\sim$49% FDM), $n \approx 3.1$, and y amounts, for example, to 10.7 kg (see also Fig. 22.16).

SUGGESTED LITERATURE

- Important aspects of various manufacturing steps are in:
  P. F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology, Vol. 1, General Aspects*, 2nd ed., Chapman and Hall, London, 1993, Chapters 5 (The syneresis of curd), 6 (Cheese starter cultures), 7 (Salt in cheese: physical, chemical and biological aspects), and 13 (Application of membrane separation technology to cheese production).
- Some trends in manufacturing processes are discussed in:
  R. K. Robinson, ed., *Modern Dairy Technology, Volume 2, Advances in Milk Products*, 2nd ed., Elsevier, London, 1993.
- Aspects of standardization and yield are extensively discussed in an IDF report:
  Cheese yield and factors affecting its control, *Proceedings of the IDF Seminar*, Cork, April 1993, International Dairy Federation, Brussels, 1994.
- A detailed discussion of factors determining yield and composition of semi-hard cheese is:
  M. G. van den Berg, G. van den Berg, and M. A. J. S. van Boekel, *Neth. Milk Dairy J. 50*, 1996, 501–540.

23

Cheese Ripening and Properties

The maturation of cheese includes all of the chemical changes occurring in the cheese after manufacture. Some alterations begin before the curd making is finished. The structure and composition of the cheese change; hence its organoleptic properties. Biochemical, microbiological, as well as purely chemical and physical aspects are involved. Development of cheese properties, including consistency and flavor, is especially attributable to the conversion of lactose, protein, fat, and, in some cheeses, citrate.

23.1 LACTIC FERMENTATION

General aspects of lactic fermentation are treated in Sections 11.1 and 11.2. Manufacture and maturation of cheese seem to be impossible without lactic acid bacteria; in most but not all cases, they are added to the milk as a starter. Mechanical inclusion in the curd (concentration factor 5–12) and growth lead to 10^9–10^{10} bacteria per gram of curd. Since cheese milk is inoculated at a level of, say, 10^7 starter bacteria per milliliter, it implies that in the fresh cheese starter bacteria replicate only a few times. Furthermore, growth in cheese stops at a fairly high pH, e.g., 5.7; the actual pH depends on the species and the strain of bacteria involved. A full explanation of these phenomena is lacking. Although the growth stops, fermentation continues, further decreasing the pH. The lower the pH and the temperature, and the higher the salt content, the slower the decrease in pH. Growth of most of the lactic acid bacteria is also slowed, if not stopped, by the salt. Accordingly, the number of these bacteria in the cheese should be high before the salting starts. (This does not hold for cheese varieties made of cheese

milk to which a high salt content is added, which contain fairly salt-tolerant lactic acid bacteria.)

Starter bacteria may vary widely as to their growth rate, the number to which they grow in cheese, and the rate at which they lose viability during ripening. This is illustrated in Figure 23.1. In addition to starter bacteria, other lactic acid bacteria may grow in the cheese and these often cause defects (Sections 24.3–24.4, 24.7).

Usually, rapid acid production is desirable in cheese that is brined in order to keep the time from molding to salting of the cheese as brief as possible. When the cheese is salted at the curd stage the curd making takes far longer (because much acid should have been produced in the curd before salt is added) and a rapid acid production is even more essential. The rate of acid production by the starter thus should be high. A satisfactory process control also demands that the cheese always acidify at the same rate because a variable rate leads to variation in syneresis and, hence, water content of the cheese (see also Sections 22.2 and 25.1).

The formation of lactic acid from lactose is paramount for the preservation of the cheese, i.e., prevention of growth of undesirable microorganisms. This conversion causes a low pH, thereby a high concentration of undissociated lactic acid and a considerably reduced lactose content. In many varieties of cheese, especially hard cheeses and those in which the curd is washed, lactose may be
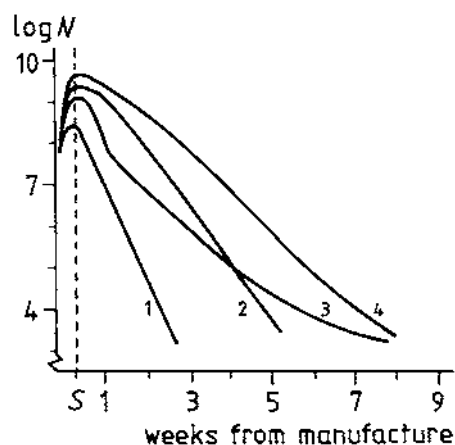
FIGURE 23.1   Number N of viable starter bacteria (CFU per g of cheese) from various starters (1–4) during the manufacture and maturation of Gouda cheese. S is the moment at which brining starts. After F. M. W. Visser, *Neth. Milk Dairy J.* **31** (1977)120–133.

completely fermented. All of these factors inhibit numerous microorganisms. An additional factor may be the anaerobic environment because the slow diffusion rate of oxygen into the cheese allows the enzyme system of the lactic acid bacteria to keep the redox potential of the cheese at the low level arrived at during the lactic acid fermentation. This effect is enhanced by the presence of a closed rind.

Starter bacteria considerably affect, either directly or indirectly, other properties of cheese such as its body (e.g., formation of holes), consistency, and flavor (see also Sections 23.5 and 23.6). Hole (eye) formation can be caused by lactococci that produce CO_2 in the fermentation of citrate (Section 11.1.3).

23.2 ENZYME SOURCES

Proteolytic and lipolytic processes are paramount to obtain the characteristic properties of ripened cheese; the relative importance of any of these processes may vary widely with the type of cheese considered. The responsible enzymes can be classified into the following groups, based on type of substrate and way of attack:

a. Proteolytic enzymes (EC 3.4), subdivided into
   1. Endopeptidases or proteinases, which hydrolyze proteins to peptides.
   2. Exopeptidases, which split peptides into smaller ones and amino acids; this group includes aminopeptidases, carboxypeptidases, di- and tripeptidases.
b. Enzymes that decompose amino acids produced by the exopeptidases, i.e., decarboxylases, deaminases, transaminases, demethiolases.
c. Lipases (EC 3.1.1), which break down triglycerides into free fatty acids, di- and monoglycerides.
d. Enzymes that break down fatty acids or their derivatives, i.e., dehydrogenases, decarboxylases.

Potential enzyme sources in cheese are:

a. Rennet enzymes, insofar as they are transferred to the cheese during manufacture and remain active.
b. Endogenous milk enzymes, especially proteinases and lipoprotein lipase. The latter enzyme is largely inactivated by pasteurization.
c. Lactic acid (starter) bacteria.
d. A flora on the surface (e.g., white molds, salt-tolerant bacteria, etc.) or in the interior (e.g., blue molds).
e. Remaining nonstarter bacteria, originating in the raw milk and surviving pasteurization.
f. Extracellular proteinases and lipases originating from psychrotrophic

bacteria growing in the raw milk. The bacteria are generally killed by pasteurization, but many of their enzymes are highly heat-resistant.

g. Recontaminating organisms in the milk and undesirable organisms growing on the cheese rind (Section 22.5).

Rennet and milk proteinases cause proteolysis; other enzyme sources may also contribute to conversion of amino acids and to lipolysis (Section 23.4).

The enzyme system of a microorganism comprises several enzymes which, depending on the location in the cell, are classified as:

- Extracellular enzymes secreted into the substrate by the intact cell
- Cell wall–associated enzymes
- Cell membrane–associated enzymes
- Intracellular enzymes exposed to the substrate after lysis of the cell

The activity of an enzyme in cheese naturally depends on the enzyme concentration. Obviously, for microbial enzymes the final number of organisms in the cheese is an essential parameter, as is lysis of the cells. Moreover, the activity of all enzymes depends on the conditions in the cheese, which may alter significantly during maturation. The following are important parameters.

a. *pH*. Every enzyme has an optimum pH at which its activity is highest.
b. *NaCl content* of the moisture in the cheese. NaCl at fairly low concentration activates certain enzymes but inhibits others.
c. *Ripening temperature* of the cheese. Under normal conditions, activity increases with temperature. The effect of temperature is stronger for lipolysis than for proteolysis.
d. *Water content* of the cheese. This affects the composition of the cheese moisture (e.g., the calcium ion activity) and the conformation of proteins. This means that the enzyme activity may depend on the conformation of the enzyme and, for proteolytic enzymes, on that of the substrate. The lower the water content, the smaller are the diffusion coefficients, which may affect reaction velocities. Generally, protein degradation is faster for a higher water content.

Note, however, that in practice most of the above parameters (especially a and d) may also affect the concentration of some of the enzymes in the cheese.

23.3   PROTEOLYSIS

During the maturation of cheese the protein is broken down by proteolytic enzymes into several products, ranging from large peptides to free amino acids and even ammonia. The ratio of the respective products may vary widely according to the type of cheese considered. In this respect the concepts of *width* and *depth*

of the ripening are being used. The width reflects the proportion of the protein molecules that is decomposed, predominantly into larger peptides. The depth refers to the extent in which these breakdown products are degraded into smaller components, among which are amino acids.

23.3.1 Methods of Characterization

Several methods are available to assess proteolysis and these may lead to widely varying results. The cheese may be extracted with water, with a solution of NaCl, $CaCl_2$, or at pH 4.6, i.e., roughly the isoelectric pH of paracasein. The extracts so obtained may be centrifuged or filtered and analyzed for nitrogen. These ''soluble-N fractions'' are heterogeneous in composition and may even contain intact protein, according to pH, ionic strength and Ca^{2+} activity of the solution. The estimated N content provides no information about the character of the proteolysis because identical amounts of ''soluble N'' of various cheeses may be composed of widely varying substances. To obtain a better understanding, the extracts are fractionated. Less heterogeneous ''NPN extracts,'' mainly containing low-molar mass peptides and amino acids, are obtained by treating the extracts with trichloroacetic acid (TCA) solutions (e.g., 12%) or with 70% aqueous ethanol; amino acids remain dissolved after a treatment with phosphotungstic acid. Amino acid nitrogen can be determined using the Kjeldahl method, but the TCA or ethanolic extracts also contain di- and tripeptides. It is possible to estimate the individual free amino acids by ion exchange chromatography, which leads to lower values of amino acid N. Free amino groups can be determined with trinitrobenzenesulfonic acid or with *o*-phthalic dialdehyde. Ammonia nitrogen can also be determined spectrophotometrically. By applying electrophoresis and chromatography (gel filtration or ion exchange, mostly by HPLC), the fractionated extracts can be used to further characterize the proteolysis. By means of quantitative electrophoresis the degradation of casein to larger peptides can be quantified.

The proteolysis can best be characterized if various methods are applied simultaneously.

23.3.2 Milk Proteinases

Introductory information is given in Section 2.5. In cheese, these enzymes decompose α_{s1}-, α_{s2}-, and β-casein. Alkaline milk proteinase is identical to blood plasmin. In milk it is predominantly found as the inactive plasminogen, with only a small percentage being active (see Section 2.5.2). At the pH of milk the enzyme has a marked affinity for casein, whereas it dissociates from casein at low pH. It is especially active at high pH and decomposes β-casein much faster than α_{s1}-casein.

An acid milk proteinase also occurs, which is less important. Its optimum pH in cheese is pH 5.1–5.6. β-Casein is decomposed more slowly than α_{s1}-casein.

Milk proteinases are not inactivated by pasteurization of milk. In addition to pH, the following are important factors affecting the enzyme activity in cheese:

a. *The proteinase content of the milk, the concentration of plasminogen activators, and the concentration of inhibitors of plasminogen activation.* All of these can vary among milkings of one cow and among individual cows.

b. *The heat treatment of the cheese milk.* In raw milk the activity of alkaline milk proteinase is less than in low-pasteurized milk, which may be ascribed to partial inactivation of compounds that inhibit plasminogen activators, resulting in a greater amount of plasmin.

c. *The scalding temperature during curd making.* High temperatures, as applied in the manufacture of several Swiss and Italian cheese varieties (Section 25.4), considerably enhance plasmin activity in the cheese.

d. *The salt content.* A low salt content in the cheese moisture (say, 2%) has a stimulating effect.

e. *The ripening temperature.*

The conditions in cheese, especially the pH, often are unfavorable for a significant proteinase activity. A marked action may, however, occur in types of cheese with a high pH, e.g., Camembert (pH 6–7), and in types with a relatively high pH and a long ripening time, e.g., Emmentaler.

The proteolysis by milk proteinases increases the amount of soluble N compounds, mainly consisting of low-molar mass peptides, MW <1400; the production of amino acids is small (see Table 23.2).

23.3.3 Rennet Enzymes

These enzymes, which have a specific function in milk clotting (Section 21.3), also have a considerable effect on the proteolysis in cheese and the ensuing properties of the product. The action of calf rennet (consisting of chymosin and 15% to 20% pepsin, as calculated on clotting activity) greatly depends on the amount of rennet retained in the cheese. The following factors affect this amount (see also Fig. 22.9):

1. The *amount of rennet* added to the milk.

2. *The pH during curd making.* The lower it is, the more calf rennet is adsorbed onto the paracasein. Hence, factors having effect are initial pH of the milk, rate of acid production by the starter, percentage of starter added, addition of $CaCl_2$, and preacidification of the milk as, for example, in the manufacture of Camembert.

3. *The scalding temperature of the curd.* At a high scalding temperature, e.g., 55°C as is applied for Emmentaler cheese, rennet (chymosin) is

for the greater part inactivated. At low pH, however, chymosin is more
heat-resistant (see Fig. 21.2).

Still other factors can be essential. Intense heating of cheese milk, using less curd
washing water, and adjusting a higher water content in the cheese have been
reported to cause a higher content of rennet in the curd.

The effect of the rennet content of cheese on the rate of proteolysis is
illustrated in Figure 23.2. The results also indicate that the rennet retains most
of its activity during the ripening. A relatively large part of the formed soluble
nitrogen compounds consists of products with a molar mass above 1400 (Table
23.2). Obviously, the depth of ripening is small, which is also expressed by the
fact that few if any amino acids form, irrespective of the rennet content of the
cheese.

The optimum pH for calf rennet action in cheese is about 5. α_{s1}-Casein
degrades rapidly; β-casein far more slowly. Moderate salt contents, up to about
4% NaCl in water, favor the degradation of α_{s1}-casein, whereas higher levels
slow it down; the degradation of β-casein is slowed down already at low salt
contents.
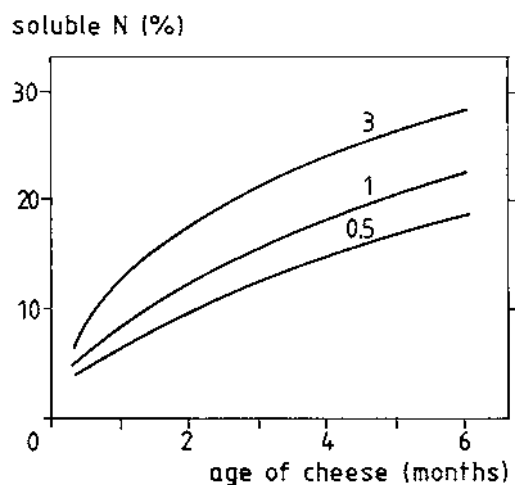
FIGURE 23.2 Proteolysis by calf rennet in aseptically made, starter-free Gouda
cheese. Production of soluble N, expressed as a percentage of the nitrogen
in the cheese. The soluble N is present in a filtrate obtained by extracting the
cheese with a 0.037 M $CaCl_2$ solution of pH 7.5, temperature 30°C. Rennet con-
tent of the cheese is ''normal'' (1, corresponding to about 300 μl/kg cheese),
half (0.5), and three times (3) the normal amount, respectively. Approximate
examples after F. M. W. Visser, *Neth. Milk Dairy J.* **31** (1977)210–239.

The rate of proteolysis by calf rennet is little affected by the ripening temperature of cheese; at, say, 4°C it differs not much from that at 14°C. Since the enzyme is more active (on synthetic substrates) at higher temperatures, it must be that the substrate in cheese is more prone to attack at lower temperature.

Shortage of calf stomachs as well as an increasing production of cheese have resulted in the use of calf rennet substitutes. Bovine pepsin, blends of porcine pepsin and calf rennet, and preparations of the molds *Mucor pusillus* and *Mucor miehei* are among those being used industrially. The properties of the cheese made by using a substitute should not differ significantly from the reference cheese. To achieve this, the means of manufacture must often be adapted because the rennets differ from calf rennet in several respects, i.e.:

a. *Susceptibility to temperature, Ca²⁺, and pH during the clotting*. Porcine pepsin, for instance, inactivates rapidly above pH 6.5. A high rennetability is paramount, as is formation of a firm gel that gives sufficient syneresis.

b. *Resistance to heat*. Too high a resistance might cause problems in the processing of whey.

c. *Distribution of enzyme activity between curd and whey*. For instance, this distribution is not affected by the pH for some mold rennets.

d. *Proteolytic activity*. Too great an activity during clotting of milk leads to a loss of yield and to excessive and abnormal degradation of the caseins during ripening, causing defects in consistency and flavor, e.g., a bitter flavor. Generally, microbial rennets are more proteolytic than calf rennet (see Fig. 23.3).

Bovine chymosin can also be produced by genetically modified microorganisms and used for cheese making. Its action is virtually identical to that of calf rennet.

23.3.4 Enzymes of Lactic Acid Bacteria

Proteolytic enzymes of the starter bacteria (i.e., mesophilic *Lactococcus* spp. for most cheese varieties) play a key role in the maturation of all ripened cheeses without a surface flora or internal blue molds. These enzymes are responsible for the ''depth'' of the proteolysis: they attack the large peptides formed by rennet or plasmin action to produce the small peptides and free amino acids that contribute most to flavor, be it directly or as precursors of specific flavor compounds. Moreover, any variation in proteolysis within a type of cheese is for the most part due to variation in activity of enzymes of the starter bacteria. Although we have a reasonable understanding of the factors determining the activity, a more quantitative and detailed knowledge is largely lacking.

Some of the *Lactoccocus* strains are proteinase-positive (Prt+), which means that they contain a cell wall–bound proteinase, which is needed for the
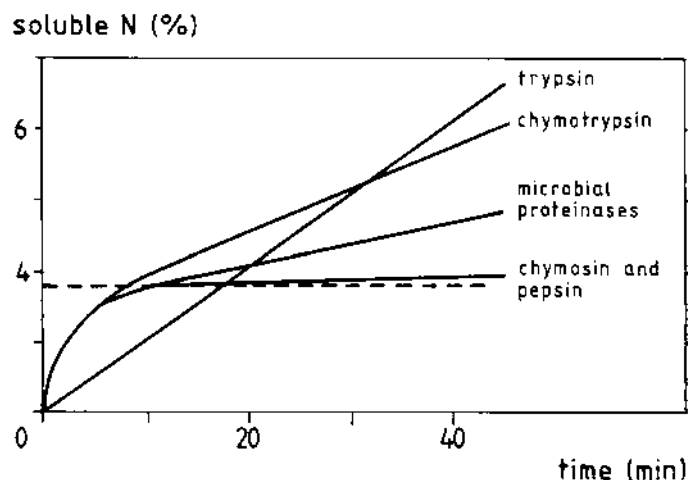
soluble N (%)

FIGURE 23.3 Splitting soluble N compounds off casein (in percentage of ca-
sein N) by various rennet enzymes. Approximate examples; high enzyme
concentration. Largely after C. Alais, *Sciences du Lait*, 3rd ed., 1974.

bacteria to grow in milk. Other strains are proteinase negative (Prt−), and these
depend on the Prt+ strains present for the formation of peptides from the milk
proteins, especially casein. In cheese much the same happens; both α_{s1}- and β-
caseins are attacked (mostly the former), but the rate of formation of peptides is
slow. However, the rennet enzymes produce peptides at a much faster rate, and
these are hydrolyzed further by bacterial enzymes. Presumably, at least part of
the peptides produced by rennet are not readily metabolized by the starter cells
unless they are broken down to smaller peptides by the bacterial proteinase. Fairly
small peptides are transported into the bacterial cell (see also Fig. 11.3) by some
specific energy-dependent transport mechanisms. The cell contains several spe-
cific peptidases that immediately hydrolyze the peptides into amino acids and
part of these can presumably diffuse out of the cell.

From the above, it would follow that only bacteria that are metabolically
active can produce amino acids because they must acquire substrate through
energy-dependent mechanisms. However, since the only energy source, i.e.,
sugar, is fully consumed within 24 h in most cheeses, this cannot be true. It is
generally accepted that lysis of the starter bacteria is needed for their intracellular
peptidases to be released in the cheese. For lysis to occur, the cell wall must be
broken down, which generally is achieved by bacterial autolysins, and subse-
quently an osmotic shock would be needed; the osmotic pressure in the liquid
around the bacterium must become either much lower or much higher than that

in the cell. Presumably, a steep salt gradient may produce this effect in cheese. It is generally believed that the lysis slowly goes on, since the peptidase activity in the cheese appears to increase during maturation. However, no lysis would occur without autolysin, and it is known that the bacteria produce autolysins only when they are growing, and that they very quickly stop growing in cheese. Another possibility may then be that the bacteria die, but retain their integrity to some extent, and that their cell envelope gradually becomes leaky, allowing peptides in the cheese to gain access to the peptidases inside the cell. Anyway, adding a significant mass of lysed starter organisms to cheese model systems considerably enhances amino acid production (if sufficient peptides are present).

The amount of enzymes produced by the starter bacteria can vary according to their growth conditions. If the bacteria are grown in a medium containing a considerable quantity of small peptides or even amino acids, they would not need the mentioned enzymes for growth, and it is indeed observed that they produce far less of them. In the present case it primarily concerns growth in the cheese milk and the fresh curd, which would involve, say, five divisions, sufficient for the bacteria to adapt to the new medium (as compared to the starter). It has been observed that growth in ''cheese milk'' that has been concentrated to a rather high degree by ultrafiltration (say, 4 times), or that has received an intense heat treatment (say, 10 min at 120°C), produces significantly reduced amounts of the cell wall–bound proteinase and of at least some of the peptidases.

All together, there is a wide variation among starter strains in terms of the extent and specificity of proteolysis. The following variables can be distinguished:

a. The *types and amounts of enzymes* that the cells can produce. Generally, growth rate and production of proteolytic enzymes are correlated. Modern fast-growing starters produce fairly large amounts of soluble N in the cheese.

b. The *growth conditions* for the bacteria (hence, pretreatment of the milk, etc.) and the dependence of enzyme production on these conditions.

c. The *number of cells* reached in the cheese. In this respect, competition between various strains, as well as proto-cooperation, may be involved.

d. The extent to which *lysis* of the cells occurs in the cheese, thereby making the peptidases accessible. This varies greatly among strains and may be one of the most important factors in determining a strain's action in cheese. Some strains can be induced to lyse by a temporary rise in temperature.

e. *Stability of the enzymes* in the cheese. Some are very stable (just like chymosin and plasmin), while others lose their activity fairly rapidly. This has been insufficiently studied.

     f.   The dependence of the *specific activity* of the enzymes on conditions like pH, salt concentration, and temperature.

Figure 23.4 gives examples of the rate of production of free amino acids.

     An important group of cheese varieties, discussed in Section 25.4, is made with thermophilic starters, generally involving mixtures of *Streptococcus thermophilus* and various *Lactobacillus* species, such as *L. helveticus, L. delbrueckii* ssp. *bulgaricus*, and *L. casei*. Although less is known about their proteolytic enzymes than about those of the *Lactococcus* species, the pattern seems to be about the same. The bacteria generally have a cell wall–bound proteinase and several intracellular peptidases. The proteolytic activity of such starter bacteria, whether they are growing in milk or are present in cheese, seems to be markedly stronger than that of the mesophilic starters. This especially concerns the lactobacilli; the streptococci can hardly produce free amino acids.

23.3.5   Enzymes of Nonstarter Organisms

Nonstarter lactic acid bacteria may enter the cheese milk by contamination and then grow out in the cheese; this generally concerns *Lactobacillus* species. Some of these do not need sugar as a carbon source but utilize certain amino acids.
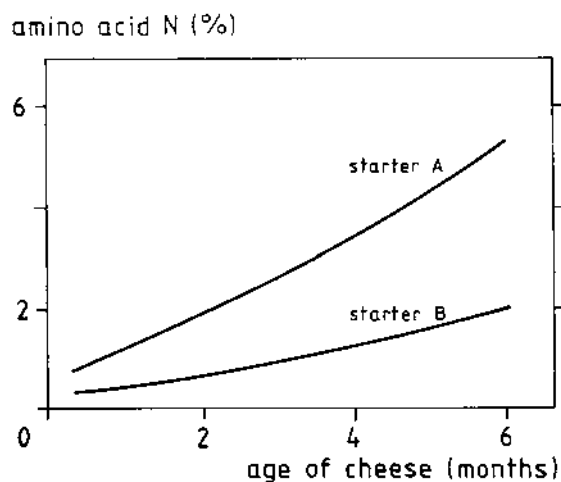
FIGURE 23.4   Production of amino acid N, expressed as a percentage of the nitrogen in the cheese, by two different starters in aseptically made, rennet-free Gouda cheese. Approximate examples after F. M. W. Visser, *Neth. Milk Dairy J.* **31** (1977)210–239.

They can reach numbers of 10^7–10^8 colony-forming units (CFU) per g of cheese. They are markedly proteolytic and can precipitate a great variety of flavors in cheese. In most cheeses these flavors are generally considered to be undesirable. Naturally, milk pasteurization and good hygienic practices during cheese making largely prevent growth of these organisms. In Cheddar cheese made of not very intensely heated milk, some lactobacilli may enhance what is considered a typical cheddar flavor, and these organisms may be added on purpose.

In cheese varieties with a specific flora, along with lactic acid bacteria several other organisms are important, including yeasts, molds, coryneforms, and micrococci (see Section 25.6). The contribution to the proteolysis and to the production of flavor compounds (Section 23.5) depends on their properties and number.

23.3.6   Interaction Between Enzyme Systems

The various enzyme sources causing proteolysis and their relative significance for protein degradation can vary widely among cheese types. Some variables are the method of cheese manufacture; cheese composition; ripening conditions, especially ripening time and temperature; and the presence of a specific flora. Examples are given in Table 23.1; see also Sections 25.3–25.6.

Proteolysis is least complicated in cheese varieties in which only milk proteinase, rennet, and starter bacteria are active, with no secondary flora involved. Protein degradation by the individual enzyme systems and by combinations thereof has been studied by means of aseptically made Gouda cheese. If all of these systems are present in cheese, the width of the proteolysis is effectively determined by the action of rennet. This holds fully for α_{s1}-casein, which is rapidly degraded at the onset of the maturation, with about 80% being decomposed within a month. Later on, the rennet enzymes cause degradation of the larger peptides produced earlier, but at a slower rate (see also Table 23.2). The α_{s1}-casein thus has for the larger part been decomposed before the action of the enzymes of the starter bacteria can be of importance. In a later stage of ripening the latter enzymes cause significant degradation of β-casein, although some 40% remains unaltered after 6 months.

All enzyme systems contribute to the production of soluble N compounds. Considering only the quantity of soluble N, the various enzymes hardly enhance each other's production; the amount of degradation products formed during the ripening corresponds roughly to the total amount produced in separate cheeses, each of which contains one individual proteolytic enzyme system (Table 23.2). However, low-molar mass components, i.e., MW <1400, reach clearly higher quantities at a combined action of all enzyme systems than could be expected from the activities of the individual enzymes, whereas the amount of components with MW >1400 is less. Clearly, the rennet action stimulates the proteolytic

TABLE 23.1 Relative Significance of Enzyme Sources for the Proteolysis in Cheese[a]

Cheese type	Rennet	Plasmin	Lactic acid bacteria		Surface flora		Internal blue mold
			Mesophilic	Thermophilic	Coryneforms	White mold	
Butterkäse, Meshanger	+++	±	++	−	−	−	−
Camembert (traditional)	++	±	++	−	++	++	−
Camembert ("modern")	+++	±	++	−	−	+++	−
Muenster	+++	±	++	−	+++	−	−
Gouda type	+++	±	+++	−	−	−	−
Cheddar type	+++	−	+++	−	−	−	−
Emmentaler	±	++	±	++	−	−	−
Gruyère	±	++	±	++	+	−	−
Provolone	±	+	−	++	−	−	−
Roquefort	++	±	++	−	−	−	+++
Gorgonzola	++	±	++	++	+	−	+++

a Approximate examples.
Significance: − no; ± a little; + some; ++ considerable; +++ very much.

TABLE 23.2   Quantity of Soluble N Compounds as Produced in Aseptically Made Gouda Cheese by the Combined and Separate Actions of Calf Rennet, Starter Bacteria, and Milk Proteinase[a]

Ripening time (months)	Proteolytic system	Soluble nitrogen, as percentage of total nitrogen				
		Total	As peptides of MW			As amino acids
			>14 000	14 000–1400	<1400	
1	Rennet	6.7	2.7	2.7	1.2	0.1
	Starter	2.5	0.2	0.6	0.4	1.3
	Milk proteinase	2.0	0.2	0.4	1.3	0.1
	All systems	12.2	1.8	2.3	6.1	2.0
3	Rennet	12.7	3.6	5.2	3.7	0.2
	Starter	4.7	0.3	0.7	1.4	2.3
	Milk proteinase	3.3	0.4	0.7	1.9	0.3
	All systems	19.5	2.3	3.3	9.1	4.8
6	Rennet	17.3	4.4	4.1	8.4?	0.3
	Starter	7.6	0.9	0.3	2.4	4.0
	Milk proteinase	4.7	0.5	1.0	2.7	0.5
	All systems	26.0	5.5	2.3	10.8	7.4

[a] Results to illustrate trends. After F. M. W. Visser, *Neth. Milk Dairy J.* **31** (1977)210–239.

system of the starter bacteria, which means that the soluble degradation products formed by rennet are decomposed to low-molar mass peptides and to amino acids.

The decrease of the amount of paracasein in a maturing cheese is a good measure for the *width* of ripening (see above), as is the amount of soluble nitrogen compounds. The *depth* of proteolysis can be defined as the ratio of the amount of low-molar mass degradation products (e.g., amino acids or products with MW <1400) to the total amount of degradation products. In most cheeses, therefore, the width of the proteolysis is mainly determined by the activity of the rennet and the depth by that of the starter bacteria.

Probably, milk proteinases, calf rennet, and starter proteinases do not (or do so insignificantly) attack undenatured serum proteins and para-κ-casein in cheese.

Maturation of cheese alters the conditions, especially the pH; the change affects the action of enzymes and their interactions. Proteolysis, especially deamination (formation of NH_3) and decarboxylation of amino acids, as well as decomposition of lactic acid, causes an increase in pH. In most cheese varieties, the pH increases by only a few tenths of a unit, in those with a distinct degradation of lactic acid and of protein it increases markedly (see Section 25.6 and Fig. 25.3). Some microbial defects also cause a considerable pH increase (Section 24.2).

When making cheese from milk concentrated by ultrafiltration to a consid-

erable degree of concentration (say, by a factor of 5), proteolysis and the development of flavor and consistency are markedly slowed. The causes are complex, manifold, and insufficiently understood. One cause is ''dilution'' of the casein by serum proteins, which are not prone to proteolysis. Furthermore, rennet activity is decreased. This means that for the same concentration of chymosin in the cheese, less proteolysis occurs. It appears that some proteins or peptides can lower chymosin activity. A third factor appears to be the decreased production of peptidases by starter organisms growing in ultrafiltered milk; this is discussed in Section 23.3.4. Finally, it may be that the starter organisms are less prone to lysis in the cheese, but this has not been clearly shown.

23.4   LIPOLYSIS

Among the enzymes that may contribute to lipolysis are the following:

1. *Milk lipase* (lipoprotein lipase) (see also Section 3.1.5). The concentration of active enzyme closely depends on the pasteurization process (see Fig. 14.2). Under the usual pasteurization conditions some 10% to 15% of the enzyme is left. In raw milk cheese the enzyme is relatively active and it may eventually increase the acidity of the fat to some 20 or 30 mmol per 100 g fat. Considering the high salt content and the low pH, it is surprising that the enzyme can be active at all in cheese, albeit slowly. The optimum pH for the enzyme is above 8; the minimum is noted to be 6. In cheese, however, lipolysis often is stronger as the pH gets lower. That can be partly, but not fully, ascribed to an inadequacy of the analytical methods. At lower pH more of the water-soluble, short chain fatty acids are dissolved in the fat and subsequently titrated. Incidentally, for identical concentrations of fatty acids, the flavor caused by fatty acids is more pronounced as the pH of the cheese is lower.

   Lipolysis considerably increases with temperature, at least in raw milk cheese. Homogenizing the cheese milk, or a part of it, or changing the surface layer of a part of the fat globules in another way, results in a considerable increase in lipolysis, albeit only for a short time, i.e., a few days. Subsequently, the rate of lipolysis appears to return to its normal (low) level. Hence, the acidity of the fat in the cheese can be adjusted as desired. In cheese made of pasteurized unhomogenized milk, with no additional lipolytic enzymes, the fat acidity does mostly not exceed 1 or 2 mmol per 100 g fat.

2. *Lipolytic enzymes*. Sometimes these are added, e.g., to ripened cheese with pasta filata. Also the rennet may include these enzymes (calf rennet does not).

3. *Microbial lipases*. Potential sources are:
   a. The flora of the milk, especially psychrotrophic bacteria in cold-stored milk.
   b. Organisms constituting a specific surface or internal flora in some cheese varieties. Lipolysis can be considerable, especially in blue cheeses (see Section 25.6).
   c. Lactic acid bacteria. These organisms are not very lipolytic. They hardly decompose triglycerides, but do mono- and diglycerides somewhat more. Because of this, the contribution of these bacteria to lipolysis mainly depends on the activity of lipases of an additional source.

23.5 DEVELOPMENT OF FLAVOR

23.5.1 Description

A cheese of satisfactory flavor always contains many different flavor compounds, among which a good balance exists. In curd, weak flavor compounds prevail, originating from the fat and some other milk components. The initial sweetness in the taste of curd, due to lactose, disappears quickly. The lactic fermentation is responsible for the acid taste, characteristic of almost all cheese varieties. In fresh-type cheeses aroma compounds formed by the starter bacteria (e.g., diacetyl) can play an important role. In ripened cheeses the salt is also an essential flavor component; the concentration varies widely among cheese types. The saltiness of the cheese is roughly proportional to its salt content rather than to its salt-in-water content.

Large changes in flavor develop during maturation. Protein has no flavor, but many degradation products have. Free amino acids and short chain peptides contribute to the basic flavor of cheese. These compounds have specific tastes: sweet, bitter, and broth-like in particular. The stage of maturation largely determines the intensity of the basic cheese flavor. The cheese may develop a bitter flavor if the protein is degraded in such a way that many short chain hydrophobic peptides are formed.

The protein degradation also greatly affects the cheese consistency and thereby the mouth-feel. Probably, the degradation affects the flavor perception (release of flavor compounds). Carbon dioxide, although without flavor per se, appears to affect the cheese flavor. Loss of CO_2 may contribute to the rapid loss of typical flavor of grated cheese.

Fat plays an essential part in the flavor of cheese, albeit an indirect part. Reducing the fat content of several well-known ripened cheese varieties results in a much less satisfactory flavor perception, even if flavor compounds associated with the fat as such contribute little to the flavor. Probably, the distribution of

aroma compounds over the fat and aqueous phases enhances a balanced flavor. The most important flavor compounds originating from the fat are the free fatty acids formed by lipolysis. The acids cause a somewhat pungent flavor. A very strong flavor may be obtained if free fatty acids develop together with flavor compounds from protein degradation. In cheese lacking sufficient basic flavor from proteolysis, free fatty acids are considered undesirable because they then produce a soapy/rancid flavor.

Mature cheese contains small amounts of several essential volatile flavor compounds. The compounds are predominantly degradation products of amino acids, including NH_3, various amines (in cheeses with a surface flora), methional (e.g., in Cheddar cheese), H_2S, phenylacetic acid, and related compounds (cheese with growth of *Brevibacterium linens*). Furthermore, the following components have been indicated: aldehydes, primary and secondary alcohols and their esters, short chain fatty acids, δ-lactones. In strongly flavored cheeses, blue-veined cheese in particular, methylketones are predominant, formed largely by microbial fat degradation. Propionic acid bacteria form propionic acid, which has a sweet taste, especially at a higher pH.

Finally, several off-flavors may occur such as yeasty flavor (alcohols and esters), unclean flavor, H_2S-like flavor, burnt flavor (by some lactic acid bacteria), cabbage-like flavor (by other lactic acid bacteria), bitter flavor (certain peptides), soapy/rancid flavor (free fatty acids), and many others (see also Chapter 24). In any individual case a detailed examination may be necessary to find out cause and remedy.

23.5.2 Changes During Maturation

Numerous components originate from lactose fermentation, lactic fermentation, decomposition of lactic acid, proteolysis, and lipolysis. *Proteolysis* at first leads to formation of amino acids and peptides, including some *bitter peptides*. In cheeses ripening under the influence of rennet and starter bacteria, a bitter flavor mainly develops if the rate of formation of bitter peptides by rennet and the rate of degradation of these peptides by enzymes of the starter bacteria are not well balanced. The capacity of lactic acid bacteria to form bitter compounds varies, and so does their capacity to decompose bitter peptides. Accordingly, the starters are classified as ''bitter'' and ''nonbitter'' starters. In other varieties of cheese, a secondary specific flora (Section 25.6) can also cause bitterness, e.g., *Penicillium camemberti* in Camembert cheese. In these cheeses, often having a high pH, plasmin can also contribute to the bitter flavor.

The desirable extent of lipolysis depends on the type of cheese concerned. For example, in Gouda and Cheddar-type cheeses a rather weak lipolysis is acceptable and even desirable, but too strong a lipolysis leads to a soapy/rancid flavor. Extensive lipolysis is characteristic of blue-veined cheese. However, the

cheese does not turn soapy/rancid. Obviously, its strong, ''deep'' proteolysis masks the flavor of the fatty acids. Free fatty acids can also originate from the fermentation of lactose and lactic acid (for example, acetic acid and butyric acid, respectively) and from the decomposition of amino acids (see below).

Several components, especially peptides, amino acids, salt, lactic acid, and, possibly, free fatty acids, give cheese a certain kind of basic flavor (Section 23.5.1). On top of that, specific aroma compounds, or the above-mentioned components when present in high concentrations, give the cheese a specific flavor. The specific compounds can be obtained by converting amino acids and free fatty acids to smaller molecules.

Amino acids can be converted in several ways by various enzymes (see also Fig. 23.5):

   a. *Decarboxylases and deaminases*. Decarboxylation of an amino acid results in the formation of CO_2 and an amine (see also Section 23.8).
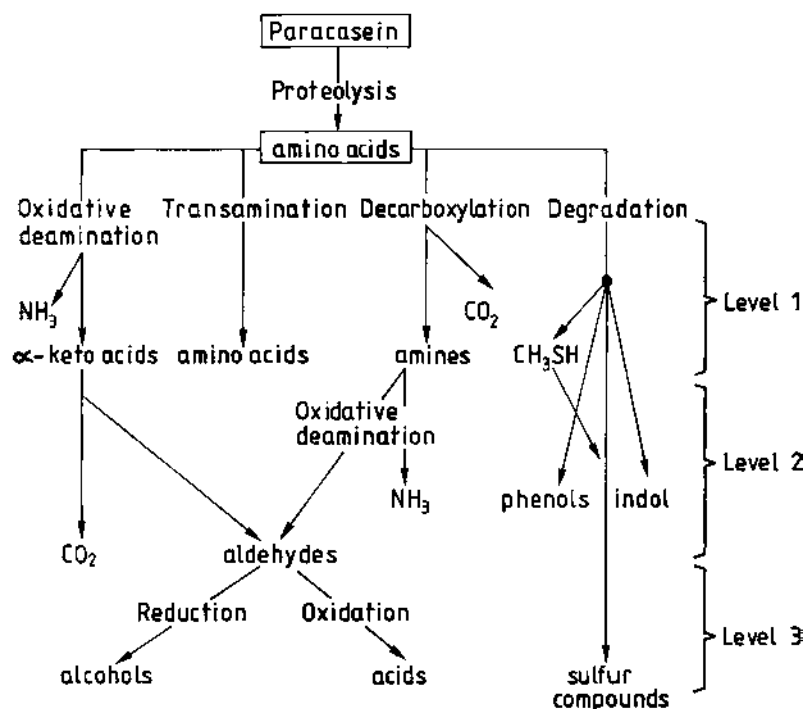
FIGURE 23.5   Diagram of the decomposition steps of amino acids during the ripening of cheese. Adapted from D. Hemme et al., *Science des Aliments* **2**(1982)113.

     Oxidative deamination of the latter component leads to NH_3 and an aldehyde from which a primary alcohol or an acid can be formed. An aldehyde can also be formed by oxidative deamination of an amino acid and subsequent decarboxylation of the resulting α-keto acid.

  b. *Transaminase*. It transforms one amino acid into another. Because of this, certain amino acids are present in cheese but not in casein. Likewise, other amino acids can be formed by hydrolytic splitting, e.g., arginine + H_2O → ornithine + urea, by arginase.

  c. *Demethiolase*. Demethiolase decomposes sulfur-containing amino acids. Methionine is a main precursor. Breakdown products include methanethiol (CH_3SH), dimethylsulfide, dimethyldisulfide, and thiapentanes or thiahexanes, e.g., 2,4-thiapentane (CH_3-S-CH_2-S-CH_3). H_2S can also be formed.

  d. *Lyase*. Most characteristic are the decompositions of tyrosine to phenol, and of tryptophan to indol, NH_3, and pyruvic acid.

The following are important conversions of *fatty acids*:

  a. β-Oxidation and decarboxylation of fatty acids, forming methylketones, e.g., acetone from butyric acid.

  b. Reduction of methylketones to secondary alcohols, e.g., reduction of acetone to 2-propanol.

  c. Formation of esters by esterase, especially esters of alcohol, methional, and phenol.

  d. Formation of oct-1-en-3-ol (CH_3–$(CH_2)_4$–CHOH–CH=CH_2) from esters of linoleic acid or arachidonic acid.

  e. Formation of lactones from unsaturated fatty acids.

The formation of specific flavor compounds in cheese depends on the ripening organisms involved and on their enzymes: type, concentration, activity, and interactions. This is illustrated in Table 23.3. It can be inferred that cheeses with a specific flora (Section 25.6) can have high levels of amino acids and fatty acids. Types of cheese with a surface flora of yeasts, *P. camemberti*, and micrococci show, in addition, higher concentrations of methylketones, secondary alcohols, NH_3, esters, and, above all, oct-1-en-3-ol; and cheeses with a surface flora of coryneforms, yeasts, and micrococci produce increased concentrations of amines, NH_3, esters, and sulfur compounds. The main fatty acid formed by propionic acid bacteria, in, e.g., Emmentaler cheese (Section 25.4) is propionate, which gives a sweet taste. Ca and Mg salts of free amino acids and of low-molar mass peptides are also among the compounds that produce the characteristic sweet taste of Emmentaler cheese.

     Nonenzymatic reactions can also play a certain role in the development of flavor compounds in cheese. For example, if H_2S reacts with methionine at a

TABLE 23.3 Some Groups of Flavor Compounds Formed By Specific Microorganisms, Important in Cheese Ripening[a]

Components formed	*Penicillium*		Yeasts	*Micrococcus* spp.	Coryneforms (*B. linens*)	Propionic acid bacteria
	camemberti	*roqueforti*				
Amino acids	+	+	+	+	+	+
Amines				+	+	
NH$_3$	+	+		+	+	
Sulfur compounds	+			+	+	+
Fatty acids (short chain)	+	+		+	+	+
Methyl-ketones	+	+				
Secondary alcohols	+	+				
Oct-1-en-3-ol	+					
Fatty acid esters			+	+	+	+

[a] A plus sign indicates that the organism involved forms much more of the component concerned than the other organisms, including the organisms of the normal starter flora. Amount and type of compounds vary widely among the groups of organisms mentioned, as well as among strains of an individual group.

sufficiently low redox potential (-150 to -200 mV), methanethiol can be formed.

23.6 DEVELOPMENT OF TEXTURE

23.6.1 Structure

The *microstructure* of cheese during the first few hours after molding, as observed by electron microscopy, reveals a matrix of paracasein micelles (diameter about 100 nm). The cavities in the matrix are largely filled with fat globules ($\sim$4 μm) and some whey. The moisture can still move fairly easily through the network. Within a day the matrix alters, i.e., it becomes more homogeneous. From now on, fat globules and much smaller protein particles can be seen. In a cheese of pH >5.2, particles of some 10–15 nm are observed; at pH <5.0 particles of at most 4 nm; and at intermediate pH particles of either size. Cavities filled with whey cannot be detected and any displacement of the moisture has become increasingly difficult. The cause of this change is to be found in the dissolution of

the calcium phosphate, and later on also in proteolysis. When hard cheese is kept for a long time (e.g., 4 months), the fat globules become partly fused, possibly due to enzymatic degradation of the membranes. Hence, in addition to a continuous aqueous phase, a continuous fat phase can develop. Often, crystals of free amino acids, or their salts, and crystals of calcium lactate are formed. An overview of structural elements is given in Table 23.4.

When observing a freshly made cross-section of a cheese, one perceives color and possible inhomogeneities, such as graininess or holes. In many cheese varieties, the cut surface looks yellowish, smooth (shiny), and slightly transparent; in such a case, a piece of cheese is elastic and not very firm. Other varieties, especially with a low pH or a high salt content, have a different appearance: The cheese looks white and dull or chalky, and it feels rather hard and brittle. In brine-salted varieties, a young cheese often shows the latter texture near the rind, the former texture in the center; the white rind portion then mostly disappears as the salt becomes more evenly distributed. Upon maturation, the above-mentioned differences disappear, especially in semihard and hard cheeses. In soft cheeses, one may observe a similar change in texture even stronger: At first the cheese has a low pH, and looks white and dull, whereas upon maturation it changes into

TABLE 23.4 Size and Numbers of Some Structural Elements of Clotted Milk and (Semi-)Hard Cheese[a]

Structural element	Volume fraction (−)	Number (m^{-3})	Size[b] (m)
Clotted Milk:			
Paracasein micelles	0.06	10^{20}	10^{-7}
Large cavities in network[c]	0.5	10^{16}	$5 \cdot 10^{-6}$
Fat globules	0.04	10^{15}	$4 \cdot 10^{-6}$
Cheese:			
Paracasein particles	0.4?	10^{24}	10^{-8}
Fat globules	0.25	10^{16}	$4 \cdot 10^{-6}$
Lactic acid bacteria	0.0005	10^{15}	10^{-6}
Curd particles	~1	10^{7}	$5 \cdot 10^{-3}$
Curd pieces[d] (Cheddar)	~1	10^{5}	$30 \cdot 10^{-3}$
Holes in Emmentaler	0.25	$5 \cdot 10^{4}$	$20 \cdot 10^{-3}$
Holes in Gouda	0.04	$2 \cdot 10^{5}$	$7 \cdot 10^{-3}$
Holes in Tilsiter	0.08	$2 \cdot 10^{7}$	$2 \cdot 10^{-3}$

[a] Approximate examples.
[b] Diameter of sphere of the same surface area.
[c] Network of aggregated paracasein micelles.
[d] 1.5 × 1.5 × 7 cm strips of curd, cut before salting.

smooth, yellowish, and almost liquid-like (see Section 25.6). These differences in appearance may be linked to the differences in microstructure mentioned above.

Several varieties of cheese show *holes* in the cheese mass. These holes can form as a result of an imperfect fusion of curd particles combined with inclusion of air, and are referred to as ''mechanical holes.'' Examples are Gouda cheese made from curd that is being stirred after whey drainage, inadequately pressed Cheddar cheese, and several types of cheese that are not (or are only lightly) pressed. In most cases, however, it concerns ''eyes,'' i.e., spherical holes originating from gas production in the cheese. Usually, the gas is CO_2, produced by certain starter lactococci, predominantly from citrate (Gouda cheese), by propionic acid bacteria from lactic acid (Emmentaler cheese), or by lactobacilli from amino acids. In general, the CO_2 production per se is inadequate to form eyes, if CO_2 exclusively forms from citrate. To that end N_2, or another gas, such as H_2, is also needed. The milk usually is almost saturated with air and therefore the fresh cheese with N_2 (because O_2 is consumed by the bacteria). If the milk is partly deaerated before the cheese making, eyeless cheese is generally obtained. At present, most of the cows' milk reaches a bulk milk tank through a closed circuit and it may contain relatively little air.

Apart from a supersaturation with $N_2 + CO_2$ (about 0.3 bar), nuclei are needed to form gas bubbles, but homogeneous nucleation, i.e., spontaneous formation of tiny gas cells, cannot occur. Air bubbles present in the milk disappear quickly, i.e., the larger ones rise to the surface, the smaller ones dissolve because of the high Laplace pressure in a small bubble. Presumably, some remaining strongly shrunken air cells can act as nuclei, provided that the milk is saturated with air. Alternatively, finely dispersed air can be blown into the mixture of whey and curd shortly before the molding of the cheese to obtain sufficient nuclei.

Slits or cracks rather than eyes can be formed if much gas is produced in the cheese, especially if it concerns H_2. The type of hole formed depends partly on the consistency of the cheese; see Sections 23.6.2 (Important consequences) and 25.3.2.

23.6.2 Consistency

Rheologists define the consistency of a material as its resistance to permanent deformation. In other words, it is the relation between the force exerted onto a material and its resulting flow. In actual practice, the time-dependent, thus elastic, deformation may be included as well. Usually, when speaking of the consistency of cheese, one refers to all rheological and fracture properties.

Cheese consistency varies widely, from rigid, almost stony (e.g., old Edam), to nearly pourable (e.g., overripe Camembert), or from rubber-like, springy (e.g., Emmentaler), to crumbly, spreadable (e.g., goat's milk cheese). Moreover, the consistency may vary within a cheese: Compare rind and center,

presence of holes, acid spots, grains of crystals, etc. Such inhomogeneity makes a precise determination of the consistency difficult.

23.6.2.1 Description

Rheological properties of cheese can be determined by various means. Often, a cylindrical piece of cheese is compressed between parallel plates. This can be done in two ways. In the first, one keeps the force constant, e.g., by putting a weight on top of the test piece, and one measures the deformation as a function of time; an example is given in Figure 23.6. The force applied can be varied. In the other method, the test piece is compressed at a constant rate and one measures the force as a function of the compression. To obtain meaningful results, the force should be recalculated to a stress, i.e., force over the cross-sectional area of the test piece, which alters during compression. Also, the deformation should be expressed as the true, natural, or Henky strain $\epsilon = -\ln(h_t/h_0)$, where h is height of the test piece and $t =$ time. Examples are given in Figure 23.8. The strain rate $\dot{\epsilon} = d\epsilon/dt$ can be varied. If the test piece is a rather flat cylinder of the same diameter as the platens of the compression apparatus; if there is complete lubrication between test piece and platens, the deformation of the test piece is true biaxial elongation. In that conformation, an (apparent) elongational viscosity (stress over $\dot{\epsilon}$) can be determined; examples are given in Figure 23.7.

The behavior of cheese under stress is always *viscoelastic*, as is depicted
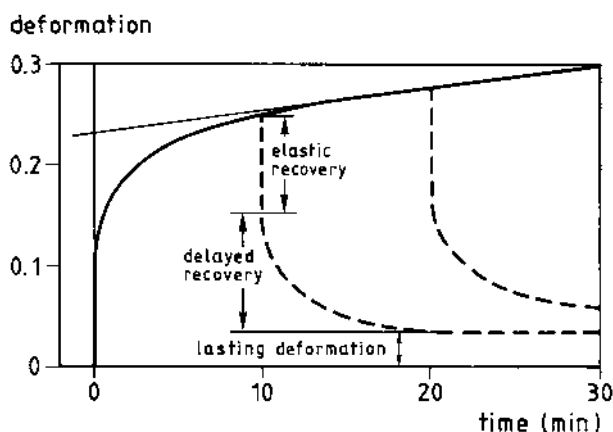
FIGURE 23.6 Example of the relative deformation of a piece of cheese after bringing it under a constant stress at time $t = 0$. The deformation after removal of the stress at $t = 10$ and 20 min, respectively, is also given (broken lines).

η'_{el} (Pa·s)
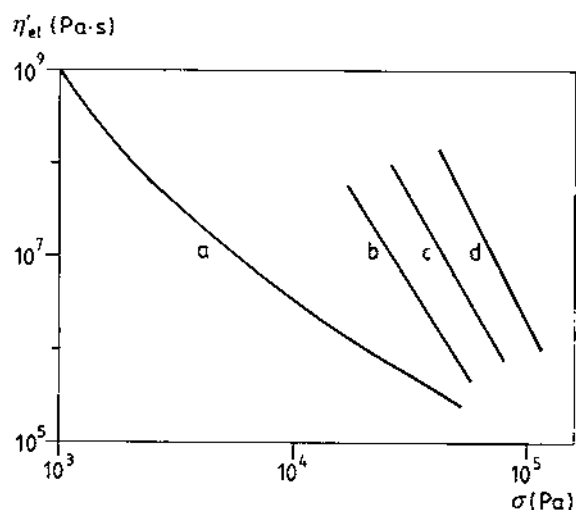
FIGURE 23.7 Influence of the stress (σ) applied on the apparent elongational viscosity (η'_{el}) of some samples of Gouda cheese (full-cream) at 20°C. (a) Age 1 week, 42% water, pH 5.21. (b) Age 1 week, 50% water, pH 4.94. (c) Age 6 months, 38% water. (d) Age 6 months, 29% water.

in Figure 23.6. At first there is a purely elastic deformation, being instantaneous and reversible. After some time there is a purely viscous deformation, which is permanent and proportional to the time during which the stress is applied. In between, the deformation behavior is more complicated. However, the magnitude of the rheological parameters (elastic modulus, apparent viscosity) and the time scale of the change from purely elastic to purely viscous vary widely. Over long time scales, viscous deformation is always predominant.

Based on other kinds of rheological measurements, both a true elasticity or storage modulus, and a true viscous or loss modulus, can be determined as a function of deformation rate. In practice, the calculation of an apparent elastic modulus E (stress divided by instantaneous deformation) and an apparent viscosity η' (stress divided by rate of the lasting deformation), from data as illustrated in Figure 23.6, can be considered satisfactory.

Most cheese has no yield stress: Even when subject to a very small stress it flows, if very slowly. Under small stresses, the elastic as well as the lasting deformation are linear, i.e., proportional to the stress. Clearly, over longer time scales, cheese behaves as a highly viscous liquid. It sags under its own weight. The apparent viscosity greatly depends on the stress applied (see Fig. 23.7). Here the elongational viscosity is illustrated. Such viscosity is relevant when the cheese

is deformed in tension, which means that a velocity gradient occurs in the direction of the deformation. This type of deformation occurs during sagging of the cheese and during eye formation.

So far we have implicitly presumed that the cohesion of the cheese mass is preserved during the deformation. At a high deformation rate, however, most cheese fractures after a certain deformation has been reached. This is illustrated in Figure 23.8A and B, where a sample of cheese is compressed until it fractures. In this way a modulus E is determined as well as a fracture stress σ_{fr} and a fracture strain ϵ_{fr}. The values of the respective quantities may vary widely among varieties of cheese: E from 10^4 to 10^6 Pa, σ_{fr} from 10^3 to $5 \cdot 10^5$ Pa, and ϵ_{fr} from 0.1 to 2. For a purely brittle material such as glass, σ is proportional to ϵ until fracture occurs and, hence, $\sigma_{fr}/\epsilon_{fr} = E$. In cheese, however, this ratio always is smaller than E ($\sigma_{fr}/E\epsilon_{fr} = 0.2$–$0.7$); the shape of the curve can vary considerably. Figures 23.8A and B show two types of behavior. A somewhat S-shaped curve (A) is observed for semihard cheese that is hardly matured (up to a few weeks old) and has a pH above about 5.15; in such a cheese, the strain at fracture strongly increases with decreasing strain rate. All other semihard and hard cheeses (lower pH and/or far more matured) show a behavior as in Figure 23.8B, i.e., the slope of the stress–strain curve keeps decreasing with strain and the strain at fracture is not or is hardly dependent on strain rate (C). The mode of fracture can also vary. Often (e.g., in case B), cracks occur in the interior of the test piece before the maximum in the compression curve has been reached; in other cases, such as in case A, the test piece strongly bulges and vertical cracks appear at its outside. Furthermore, the modulus and the stress at fracture increase slightly with
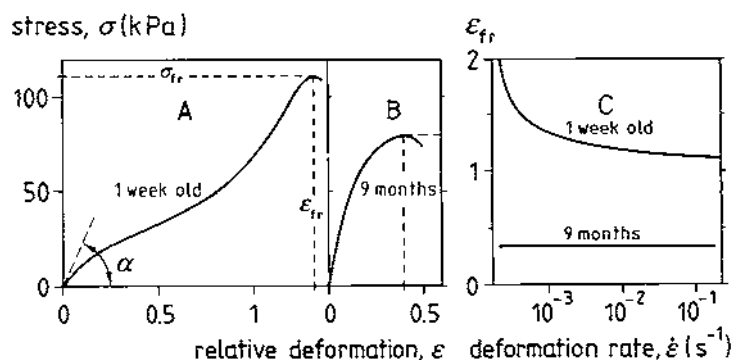
FIGURE 23.8 Fracture phenomena in Gouda cheese. (A, B) Examples of compression curves at $\dot{\epsilon} \approx 10^{-2}$ s^{-1}; tan α is the modulus (E); σ_{fr} the fracture stress and ϵ_{fr} the relative deformation or strain at fracture. (C) Examples of the influence of the deformation rate on the strain at fracture.

the strain rate. At very slow deformation, a cheese may not fracture at all but may start to flow (see Figure 23.8C, upper curve, where ϵ_{fr} goes to infinity for very small $\dot{\epsilon}$). This can often be noted in a soft cheese, which can be spread on bread, etc. Prevention of fracture within a firmer cheese is only possible at extremely slow deformation rates.

The *firmness* of cheese can be defined either as the modulus or as the fracture stress. The latter seems more appropriate because one is usually interested in deformations causing the cheese to break into pieces. Methods applied in practice to characterize the firmness of cheese often determine a quantity related to a fracture stress, e.g., the force needed to press a plunger of a given diameter at a given speed into the cheese.

In addition to the specifications ''firm'' and ''soft,'' an important characterization of the consistency of cheese is *short* and *long*. Wet sand and a rubber band are examples to illustrate the extremes on the scale of short to long. A long cheese is rubbery but not hard; a piece of it can be bent considerably before it fractures. A sample plug taken from such a cheese is thinner than the inner diameter of the cheese borer. A short cheese does fill the borer entirely because its elastic deformability is smaller. If, moreover, the latter cheese is soft, it is plastic and spreadable; whereas a firm and short cheese is stiff and almost crumbly. The properties short and long obviously are linked to the cohesion forces between cheese ''particles.'' Shortness is best defined in terms of the deformation needed for fracture, thus ϵ_{fr} (the smaller ϵ_{fr}, the shorter the cheese).

23.6.2.2 Factors Affecting Consistency

Several factors affect the consistency of cheese, but it is not easy to establish quantitative relationships: one cannot vary one factor without affecting other factors. Moreover, one factor can affect a young cheese and a mature one in different ways. Obviously, the data in Figure 23.9 have to be considered with caution. We will now discuss the effects of several variables on modulus E, deformation (strain) at fracture ϵ_{fr}, and fracture stress σ_{fr}.

    a. *Temperature*. If the cheese contains much fat, the temperature considerably affects E as well as σ_{fr}. The key factor thus is the melting of the fat. In a low-fat cheese E decreases by some 30% when the temperature is increased from 15 to 25°C, and by some 80% from 15 to 60°C.

    b. *Fat content*. From variable a it is clear that the effect of the fat content greatly depends on the temperature. Incidentally, a cheese of the same type but with a higher fat content in the dry matter usually has a higher water content in the fat-free cheese, and thus a lower E and σ_{fr}. Data in Figure 23.9 refer to identical water contents in the fat-free cheese for cheeses with 10% and 60% fat in the dry matter, respectively.

    c. *Water content*. The water content in the fat-free cheese has an over-
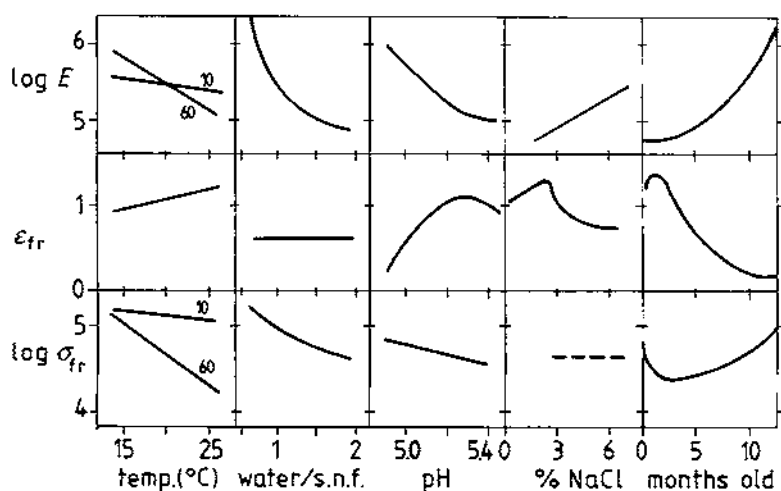
FIGURE 23.9 Rheological properties (modulus *E*, fracture strain ϵ_{fr}, fracture stress σ_{fr}) of Gouda-type cheese. Effect of the temperature (water/solids not fat ≈ 1.3; 10% and 60% fat in the dry matter, respectively); the ratio of water to solids not fat; the pH (cheese of 4 weeks old); the salt content (pH 5.2, water content 50%); and the age of the cheese.

whelming effect on *E*. Note the logarithmic scale in Figure 23.9. The network of paracasein is the main cause of the stiffness of the cheese; hence the paracasein concentration will have a considerable effect. A further decrease of the water content, e.g., by 1 percentage unit, has a stronger effect if the water content already is low. The water content hardly affects ϵ_{fr} (at least if the deformation rate is not too slow), but it can affect the fracture mode.

d. *Acidity*. The pH has a considerable effect on each of the consistency parameters and also on the fracture mode. The shortness is definitely affected. The cheese is ''longest'' at a pH of about 5.3. The relationships between pH and consistency may, however, depend on other factors (see e–g, below) and are therefore somewhat uncertain.

e. *Calcium phosphate*. The effect of the calcium phosphate content is insufficiently known because it cannot be varied independently. Presumably, however, its influence is not great unless the differences in concentration are large.

f. *Salt content*. Often, a higher salt content is associated with a far higher modulus, but the main cause may be that in practice a higher salt content mostly involves a lower water content. Even if the water content

is kept constant, as in Figure 23.9, E distinctly increases with the ionic strength. There is an abrupt and strong decrease of ϵ_{fr} at about 2.5% salt, i.e., 5% NaCl in the water. A cheese with a high salt content and a low pH is excessively short.

g. *Protein degradation.* Proteolysis has a considerable effect. However, the precise effect is poorly known because so many other factors also change when a cheese matures (see below). It is certain that the decomposition of casein causes a decrease of ϵ_{fr} and of σ_{fr}.

Figure 23.7 illustrates that the apparent viscosity of the cheese depends on the water content and maybe on the age of the cheese. It is dominated, however, by the pH, especially in young cheese and at small stresses. The viscosity markedly decreases with increasing temperature, although reliable results are scarce.

23.6.2.3 Changes During Ripening

Several changes occur. The main ones can be outlined as follows:

drying up ⟶ water content

proteolysis ⟶ ionic strength

⟶ pH
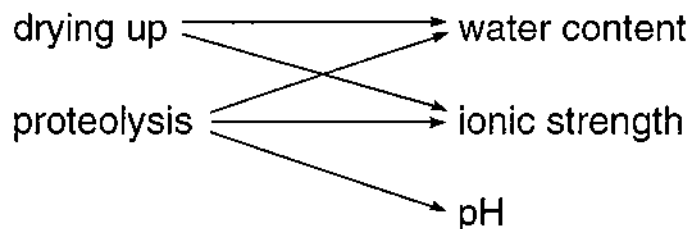
Proteolysis causes the uptake of water (it decreases the water content by, say, 1%) and the formation of ionic groups ($-COO^-$ and $-NH_3^+$). Generally, proteolysis increases the pH.

It will be clear that the changes involved, and their rates, closely depend on conditions, including size and shape of the cheese (which mainly affect the evaporation of water), amount of retained rennet, type and number of bacteria (or the enzymes released), pH, ionic strength, surface flora, and such ripening conditions as temperature and relative humidity. In Figure 23.9 examples are shown of the influence of the ripening time on the consistency of Gouda cheese. The decrease of the water content is the main cause of the increase of the modulus, whereas the proteolysis causes the cheese to become shorter. As a result, the firmness (σ_{fr}) decreases slightly in the beginning and increases substantially afterward. Organoleptic observation reveals that the young cheese is somewhat rubbery, becoming plastic after maturation.

The changes in consistency during ripening naturally depend on the type of cheese. A firm (i.e., usually a dry) cheese becomes crumbly. Cheese with a

high water content usually has a low initial pH, such as 4.8. It is short and firm. At constant pH and water content, proteolysis causes little further change in consistency (the texture becomes somewhat shorter). If the pH of the same, unripened cheese is increased to, say, 5.4, the consistency becomes rubbery; proteolysis causes the cheese to become soft and plastic, even liquid-like, at this pH. This is essentially what happens when a surface flora grows on a cheese. It consumes lactic acid and thereby increases the pH of the cheese from the rind inward, causing the cheese mass to become soft, provided that sufficient proteolysis has occurred, predominantly due to rennet action. The typical soft and almost liquid consistency develops if:

- The ratio between water and solids not fat > 2
- The pH > 5.2
- At least 60% of the α_{s1}-casein has been degraded, and
- The calcium phosphate content is not very high

See also Section 25.6.1.

23.6.2.4 Important Consequences

During various stages of making, curing, handling, and eating cheese, various rheological properties are of importance.

a. During molding, the curd grains must be sufficiently deformable to enable them to fuse into a coherent mass. Therefore, the apparent viscosity η' must be low. This causes no problems if the water content and the temperature are not too low; η' then is, for instance, 10^6 Pa · s. During classical ''cheddaring,'' the curd has to deform at a fairly high rate, the velocity gradient being $>10^{-3}$ s^{-1}. The stress applied is at most 10^3 Pa. Hence, the elongational viscosity should be $<10^6$ Pa · s. At the prevailing low pH this only holds at a fairly high temperature.

b. The cheese has to retain its shape under its own weight. Because of this, soft cheese is made flat-shaped. For several cheeses, the apparent viscosity as such is insufficient to resist considerable deformation. Consider, for example, cheese (a) in Figure 23.7. Assuming the height of the cheese to be 10 cm, the maximum stress due to its own weight (height $\times$ density $\times$ g) $\approx 10^3$ Pa. We see that the apparent elongational viscosity then is approximately 10^9 Pa · s. Consequently, the elongation rate (stress/viscosity) would be about 10^{-6} s^{-1}, or roughly 0.1 day^{-1} (10% per day). The average stress is half the maximum one and the relative change in height is about half the elongation rate, but it means that the height would decrease by a few percent per day. This does

        not normally happen and this is mostly due to the rind of the cheese being much firmer; this may, in turn, be due to its lower water content and its initially higher salt content. Still, the cheese may sag to a considerable extent, especially at high temperatures. Sometimes, cheese loaves are ''supported'' by bands, etc.

c. If more gas is produced than can dissolve in the cheese at a given overpressure, holes will form (Section 23.6.1). If the cheese mass has a low viscosity, it may ''flow'' due to the pressure in the hole, and the hole formed remains spherical, i.e., an ''eye.'' If the viscosity is high and gas production rate is high (rapid diffusion of gas to the hole), then the gas pressure in the hole will become high. If, moreover, the cheese mass has a low fracture stress (which often occurs in a short cheese, ϵ_{fr} being small), then the gas pressure in the hole becomes higher than the fracture stress and may cause fracture and formation of slits or cracks. Clearly, the risk involved is smallest if the pH is approximately 5.25, the salt content is $<2.5\%$, and only little protein has been degraded before the holes are formed.

d. Handling of the cheese must be possible, as it has to be cut, spread, or grated, according to usage. The cheese should mostly neither be too long nor too firm. Stickiness may be a problem in well-matured, soft or semihard cheeses.

e. The cheese must be edible, i.e., be readily deformable in the mouth, and preferably be rather homogeneous. A long cheese that is also firm is considered unpleasant by many consumers. It is very difficult to chew a very hard cheese (very low water and low fat content); in such a cheese, the presence of weak spots, such as those provided by cumin seeds, eases chewing considerably. Usually, the consistency in the mouth is an important quality mark.

f. Several cheese varieties must melt down satisfactorily at high temperature, e.g., cheese used on pizzas. It should flow but keep its integrity.

Items a and b relate to a slow deformation (flow) of the cheese mass, whereas d and e relate to quick deformation and fracture. Completely different rheological measurements must thus be performed to evaluate the various cheese properties.

23.7 ACCELERATED RIPENING

The quality of a cheese, as perceived by the consumer, will pass through a maximum during ripening. This is illustrated in Figure 23.10. The characteristic properties, i.e., flavor and consistency, take a certain time to develop. Subsequently, ongoing decompositions lead to undesirable flavors (e.g., a too pronounced lipol-
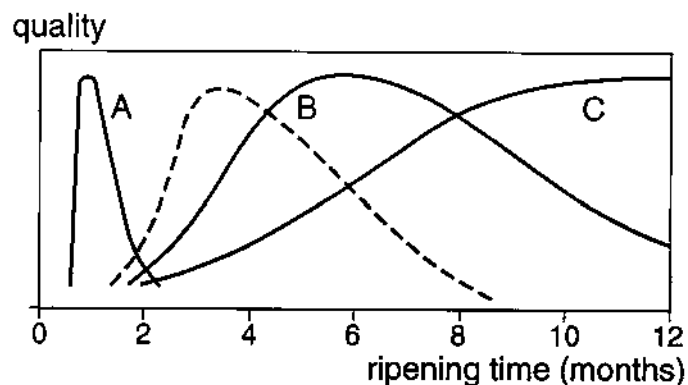
FIGURE 23.10 Approximate eating quality of cheese during the maturation. A represents, for instance, a Camembert cheese, B a mild Cheddar, and C a mature-type Gouda. The broken line is a hypothetical example of B made by applying accelerated ripening techniques.

ysis). The product may also lose quality due to evaporation of water. As a rule, the process of rise and subsequent decline proceeds faster and is more pronounced in a cheese of a higher water content (see also Section 25.1). The decline in quality can to some extent be checked by storing the cheese at a lower temperature after sufficient ripening has occurred.

Obviously, most semihard and hard cheeses need a long ripening time. Storage and maintenance of cheese are expensive because of investments in buildings and machinery, and costs of energy and labor. Reducing the ripening time is therefore attractive from an economical point of view, especially for slowly maturing cheese without a secondary flora. Conditions for an accelerated ripening process are as follows:

a. The properties of the cheese should not differ greatly from those of the reference product.
b. Overripening of the cheese should be prevented.
c. The process costs should not exceed the economic gain of shortening ripening time.
d. Legal and public health aspects should be upheld, e.g., with respect to the permissibility of enzyme preparations and their possible toxicity.

Accelerated ripening processes are primarily aimed at accelerated flavor formation (i.e., a stronger proteolysis and/or lipolysis), while maintaining a satisfactory

texture. Several methods have been tried, some of which are discussed here briefly.

23.7.1   Increase of Ripening Temperature

The main effect of an increase in temperature is based on an increased proteolytic activity of the normal starter flora. The protein degradation by calf rennet is much less affected. Furthermore, lipolysis is much more increased by an increase in temperature than proteolysis. Increasing the ripening temperature is a simple and legal method that can be applied for cheese varieties with a traditionally low ripening temperature. Cheddar cheese, for example, traditionally matured at about 7°C, could be matured at 13°C, providing the bacteriological quality of the milk is satisfactory. In the case of cheese with a higher traditional ripening temperature (about 13°C) a further temperature increase will readily cause flavor and consistency defects, e.g., bitterness and butyric acid fermentation in Gouda cheese.

23.7.2   Use of Enzyme Preparations

Lipolytic and proteolytic enzymes accelerate the production of flavor compounds. A successful use of the preparations is complicated by the need to attain a satisfactory balance among the various enzymes involved in the ripening process. An imbalance readily causes off-flavors, and too strong a proteolysis can lead to consistency defects. Because of this the lipolytic enzyme preparations are exclusively applied to cheese that is characterized by a distinct lipolysis, e.g., Italian hard cheeses. Most proteolytic preparations originate from molds and bacteria. Usually, they display endopeptidase activity that should, however, be limited to avoid such defects as bitterness and a weak consistency. The number of suitable preparations is thus limited and they can only be used in fairly low concentrations. An extract of *Bacillus subtilis*, i.e., a neutral protease, seems the most suitable. Moreover, it has the advantage of being barely active below 8°C and it is even inactivated at lower temperatures. Hence, overripening can be prevented. Flavor defects can be kept to a minimum by combining the endopeptidase activity of enzyme preparations with the exopeptidase activity of extracts of lactic acid bacteria.

The enzyme preparations can be added in various ways. Addition to the cheese milk has the advantage of resulting in an even distribution throughout the cheese. The method is, however, expensive because most of the enzyme is usually lost with the whey and because of a lowered cheese yield if strongly proteolytic preparations are used. In the manufacture of Cheddar cheese, the enzyme may be added to the curd with the salt. Enzymes may also be enclosed in or adsorbed onto various kinds of particles (liposomes, fat globules). Basically, it offers the opportunity to control the production of flavor compounds.

23.7.3 Increase of the Number of Lactic Acid Bacteria

This method, essentially leading to an increase of the enzyme capacity of the starter flora, is an obvious one: The enzymes in the bacterial cells give a balanced flavor and this balance is unlikely to be adversely affected by the number of cells. Therefore, the risk of defects is small. A higher number of bacteria cannot simply be attained by adding more starter; the resulting faster acid production would cause a more rapid syneresis, requiring adjustment of the curd-making process. The easiest way to solve the problem—also from an economical and technical point of view—is to heat-treat the starter bacteria to such an extent that their lactic acid–producing capacity is largely lost, whereas their proteolytic capacity is retained. The heat-treated starter then is added to the cheese milk, in addition to the normal starter. Instead of heat-treated starters, strains that are unable to produce acid can be added.

23.7.4 Increasing the Rate of Lysis of Starter Cells

Since it has become known that lysis of starter organisms causes intracellular peptidases (and possibly other enzymes) to become accessible, which is paramount for flavor development in many cheese varieties, an obvious method to enhance ripening rate is to enhance lysis. It is clear that starter strains vary widely in their rate of lysis in cheese, which means that proper selection of strains is desired. Some strains carry a prophage that can become an active phage by a heat shock, leading to considerable lysis. Since the heat shock has to be applied during curd making, enhanced syneresis may pose a serious problem. Some other methods to induce lysis have also been tried. All together, the practical results are still fairly uncertain. One difficulty is the uncertainty about the stage at which lysis should occur because of the largely unknown stability of the peptidases released.

23.7.5 Addition of Other Bacteria

Besides selecting highly proteolytic mesophilic starter strains, the addition of thermophilic lactic acid bacteria to cheese milk for Gouda- and Cheddar-type cheeses has been tried (see also Section 23.3.4). Some special precautions have to be taken in the manufacturing process, but accelerated ripening can certainly be achieved. However, the cheese so obtained has a flavor note that clearly differs from that of the reference cheese.

In summary, the ripening of cheese can be accelerated in various ways. With respect to proteolysis much attention is given to the formation of low-molar mass peptides and amino acids. The correlation with the development of flavor may, however, be poor. The mechanism involved in the aroma

development in cheese is insufficiently known, as is the relationship between the genetic properties of lactic acid bacteria and the production of aroma compounds.

23.8 NUTRITIVE VALUE AND SAFETY

The nutritive value of cheese follows logically from its composition: much high-quality protein and often much fat. In most cheeses, it does not vary significantly with the protein degradation. The calcium content varies widely, according to the way of manufacture, from 1 g · kg^{-1} in quarg to 10 g · kg^{-1} in Gouda-type cheese (see also Fig. 25.4). The concentration of the water-soluble vitamins B often is not high, that of vitamin B$_{12}$ may be substantial.

A negative aspect of the cheese composition can be the high salt content. Allergies caused by cheese are rarely encountered. Nitrate and nitrite contents are far below the acceptable levels, even when nitrate is added during the manufacture (see Section 24.2). The concentration of D(−) lactic acid may increase to half the lactic acid concentration, depending on the type of starter used (see Table 11.2) and on the presence of contaminating bacteria, e.g., certain pedio-cocci that can convert L(+) to D(−) lactic acid and vice versa. Apart from that, the concentration of D(−) lactic acid is always far below toxic levels. Most cheese varieties contain very little lactose, and cheese agrees well with people suffering from lactose malabsorption.

Sometimes very low concentrations of mutagenic *nitrosamines* [R$_1$-N(-N=O)-R$_2$] are found in cheese. The addition of nitrate has been held responsible, but that proved incorrect. Nonvolatile nitrosamines can form in the stomach when cheese is eaten together with nitrate-containing food, e.g., some vegetables. These compounds are, however, hardly mutagenic, and that only if they are activated. Hence, their carcinogenicity remains very doubtful. Much more dangerous are the nitrosamides [R$_1$-CO-N(-N=O)-R$_2$] which have, however, not been found in cheese. On the contrary, cheese seems to inhibit the formation of nitrosamides in the stomach. Cheese can obviously act as an antimutagenic agent and, presumably, the paracaseinate is responsible.

In cheese, amines can be formed by decarboxylation of amino acids (see Table 23.5). Since the decarboxylation is performed by microorganisms, the amines are referred to as *biogenic amines*. Several of these amines are toxic. They can cause discomfort, such as a headache or dizziness, but the susceptibility varies with individuals and may depend on the use of particular drugs. Several species of bacteria, and probably also other microorganisms, can decarboxylate certain amino acids, but in all instances it only concerns particular strains. If these strains are not present (e.g., because of pasteurization of the milk and adequate hygienic measures) no amines are formed. If, however, the decarboxylating strains (especially some lactobacilli) are present, then the production of amines is

TABLE 23.5 Biogenic Amines and Their Potential Occurrence in Many Cheese Varieties[a]

Amine	Toxic dose[b] (mmol)	Formed from	Amount of amino acid in casein (mmol · kg⁻¹)	Responsible bacteria	Highest concentration in cheese (mmol · kg⁻¹)
Cadaverine $NH_2(-CH_2)_5-NH_2$	High[c]	Lysine	560	Salt-tolerant lactobacilli, coliforms (e.g. *Hafnia alvei*)	35
Putrescine $NH_2(-CH_2)_4-NH_2$	High[c]	Arginine[d]	(220)	Salt-tolerant lactobacilli, several other lactobacilli, coliforms (e.g. *Hafnia alvei*)	11
Phenylethylamine $C_6H_5-CH_2-CH_2-NH_2$	?	Phenylalanine	330	*Streptococcus faecalis*, etc.[e]	12
Tyramine $HO-C_6H_4(-CH_2)_2-NH_2$	0.07[f]	Tyrosine	340	*Lactobacillus brevis* *Streptococcus faecalis*, etc.[e]	15
Tryptamine $C_6H_4-C_2NH_3(-CH_2)_2-NH_2$	Rather high	Tryptophan	60	Enterobacteriaceae ?	0.3
Histamine $C_3N_2H_3(-CH_2)_2-NH_2$	0.6	Histidine	190	*Lactobacillus büchneri* *Escherichia* spp. *Micrococcus* spp. Propionic acid bacteria	18

[a] The toxic level varies widely among individuals and eating habits. Of the bacteria mentioned, only particular strains can decarboxylate amino acids. The highest concentrations found in cheese are very exceptional.
[b] For sensitive persons.
[c] But it can raise the toxicity of histamine.
[d] Through ornithine.
[e] If very high numbers are present.
[f] If particular drugs are being used.

often determined by the production of free amino acids, hence by the proteolysis. Therefore, high concentrations may be found in mature cheese, often soft-type cheeses. The highest concentrations found that are given in Table 23.5 refer to blue-veined cheese. In general, fecal streptococci do not proliferate to such numbers that significant amounts of phenylethylamine form. Most attention is usually focused on histamine.

Mycotoxins can be toxic and carcinogenic. There are three potential sources in cheese:

a. *The milk*. It mostly concerns traces of aflatoxin M_1, which originate from growth of molds on the cattle feed.
b. *Functional molds*. Strains of *Penicillium roqueforti* on blue-veined cheese and of *P. camemberti* on white-molded cheese can produce toxins, which are, however, hardly toxic and are found in cheese in very small quantities at the most.
c. *Molds that grow on a cheese rind which is regularly cleaned*, mostly *Penicillium* and *Fusarium* species. Toxins are, however, not observed in cheese, although some strains are capable to form the compounds. *Aspergillus versicolor* can still grow on the rind of cheese with a latex coat, and form the slightly toxic and mutagenic sterigmatocystine. Proper cleaning of the rind is the obvious remedy.

Occasionally, *pathogenic bacteria* are found in cheese, but most of the pathogens that can occur in milk are not (see also Section 4.2). A survey is given in Table 23.6. All potentially harmful bacteria are killed by pasteurization. Most of the types cannot grow in cheese due to its low pH and high salt content. Most problems are encountered in cheese made of raw milk if hygiene during the manufacturing process is poor and the curd does not acidify adequately. Pathogenic coliforms are sometimes found in soft cheese. *Salmonella* and *Yersinia* rarely occur in harmful numbers. In all cheese varieties, including hard cheese, toxin from *Staphylococcus aureus* can be present, provided these bacteria can grow in the milk or on a moist cheese surface. *Listeria monocytogenes* is of special concern because it can grow on a cheese surface and can survive for a relatively long time. The bacterium is killed by pasteurization. It is sometimes found on the rind of soft cheese made of raw milk.

SUGGESTED LITERATURE

- Cheese ripening is discussed in:
   P. F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology, Vol. 1, General Aspects*, 2nd ed., Chapman and Hall, London, 1993, especially Chapters 10 (Biochemistry of cheese ripening) and 14 (Acceleration of cheese ripening), and part of 6.

TABLE **23.6** Pathogenic Bacteria That Can Occur in Raw Milk, and Their Potential Occurrence and Growth in Cheese (Not in Fresh Cheese)

Designation	Growth in raw milk	Survive pasteurization	Growth during cheese making	Growth in cheese (a)	Die off in cheese (b)	Ever isolated from cheese	Remarks
Brucella abortus	No	No	No	No	Yes	?	
Pseudomonas aeruginosa	Yes	No	Yes	No	0.3	?	
Aeromonas hydrophila	?	No	No	No	0.1	?	
Enteropathogenic coliforms	Yes	No	Yes	Yes/No	1–5	Yes	Can grow in/on soft cheese, pH >5.3; no enterotoxin at $T < 15°C$
Salmonella spp.	Yes	No	Yes	No	1–3	Yes	
Yersinia enterocolitica	Yes	No	?	No	Yes	Yes	
Staphylococcus aureus	Yes	No	Yes	No	1–3	Yes	Grows on moist cheese surface; toxin resists heat and proteolysis
Mastitic streptococci	Yes	No	No	No	Probably	No	
Clostridium perfringens	No	Yes	No	No	?	No	
Clostridium botulinum	No	Yes	No	No	?	?	
Listeria monocytogenes	Yes	No	Slightly ?	No	3–18	Yes	Grows on (soft) cheese; is psychrotrophic and fairly salt-resistant
Mycobacterium tuberculosis	No	No	No	No	Yes	No	
Campylobacter jejuni	No	No	No	No	0.1	No	

[a] If pH < 5.4 and some salt present.
[b] The figures represent the number of weeks needed for one decimal reduction in semihard cheese.

- A general review on accelerated ripening is given by:
  P. F. Fox, J. M. Wallace, S. Morgan, C. M. Lynch, E. J. Niland, and J. Tobin, Acceleration of cheese ripening, *Antonie van Leeuwenhoek* **70**, 1996, 271–297.
- Aspects of flavor are reviewed by:
  P. L. H. McSweeney, H. E. Nursten, and G. Urbach, Flavours and off-flavours in milk and dairy products, Chapter 10 in: P. F. Fox, ed., *Advanced Dairy Chemistry, Vol. 3, Lactose, Water, Salts and Vitamins,* 2nd ed., Chapman and Hall, London, 1997.
- Aspects of texture are treated in:
  Rheological and fracture properties of cheese, Bulletin of the International Dairy Federation No 268, Brussels, 1991.
- Nutritive value is discussed by:
  E. Renner, Nutritional aspects of cheese, Chapter 15 in: P. F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology, Vol. 1, General Aspects*, 2nd ed., Chapman and Hall, London, 1993.
- Pathogens in cheese are discussed in:
  E. A. Zottola and L. B. Smith, Growth and survival of undesirable bacteria in cheese, Chapter 12 in: P. F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology*, *Vol. 1*, *General Aspects*, 2nd ed., Chapman and Hall, London, 1993.

24

Microbial Defects

Several microorganisms growing in cheese or on the cheese surface can cause texture and/or flavor defects. The growth is determined by the following:

a. *The microbial composition of the milk*. Important aspects are:
   1. *The hygienic measures during milking*. Satisfactory hygienic standards are paramount, especially for cheese made from raw milk.
   2. *Pasteurization of the milk*. Low pasteurization kills most of the spoilage organisms, with the exception of the spores of clostridia, especially butyric acid bacteria, and of some types of streptococci (Section 6.3.3).
   3. *Bactofugation*. This process can considerably reduce the microbial population, as well as the number of *Clostridium* spores.
   4. *Recontamination of the milk*.
b. *Measures to inhibit contamination* during manufacture and maturation of the cheese. Regular cleaning of the equipment, treatment of brine, and overall hygiene are important.
c. *Measures to limit the growth* of microorganisms and its damaging effects during manufacture and maturation of the cheese. Adding inhibitors to the cheese milk, enhancing a rapid and complete fermentation of lactose, and proper treatment of the cheese rind will limit the growth considerably. Incidentally, microorganisms in the milk, including spoilage organisms, are almost completely entrapped in the curd; this entrapment raises their number per gram of cheese by a factor of 10 as compared to the number per gram of clotted milk.
d. *The physicochemical properties* of the cheese and ripening conditions.

> In particular, lactic acid content and pH, content of NaCl, presence of sugars, ripening temperature and time, and relative humidity of the air determine whether or not microorganisms can grow in or on the cheese.

The above-mentioned aspects will be discussed further below. Although this overview is basically valid for all cheese, it focuses on cheese varieties without a specific surface flora or an internal flora. Table 24.1 gives a survey of deteriorative organisms. Fermentation by coliform bacteria causes an excessive gas production at an early stage of cheese manufacture, i.e., before or during brining. The defect is referred to as ''early blowing,'' as opposed to ''late blowing,'' which refers to fermentations becoming perceptible after a longer keeping time.

The influence of the rate of gas production and of the type of gas produced (hydrogen gas is feared most seriously) on the development of texture defects, as well as the relationship between the shape of the holes and the consistency of the cheese, is dealt with in Section 23.6. It should be stressed that microbial spoilage of cheese does *not* imply that the cheese presents a health hazard. This is discussed in Section 23.8.

24.1   COLIFORM BACTERIA

Coliform bacteria rarely cause defects in cheese made of pasteurized milk because they are not heat-resistant. In practice, however, a slight recontamination of pasteurized milk by coliform bacteria of nonfecal origin, e.g., *Enterobacter aerogenes*, is inevitable. To restrict their growth, satisfactory hygienic measures during cheese manufacture have to be maintained.

These organisms can only grow as long as sugar is available for fermentation because they cannot ferment lactic acid. Depending on the extent of recontamination they can rapidly proliferate to reach considerable numbers during cheese making if temperature and pH are favorable. The main metabolites formed are CO_2 and H_2, and, to a lesser extent, lactic acid, acetic acid, succinic acid, formic acid, ethanol, and 2, 3-butylene glycol. As a result, flavor defects develop in the cheese, including yeasty, putrid, and gassy flavors. This is also partly due to some strains attacking protein degradation products.

The growth of coliforms does not necessarily cause texture defects (early blowing) because development of such defects depends on the ability of strains to ferment citric acid. Some strains, especially *Enterobacter aerogenes*, ferment citric acid slowly, whereas others, like *Escherichia coli*, do so more quickly. Rapid fermentation reduces the risk of blowing because the produced H_2 reacts with metabolites of the citric acid fermentation. Formation of off-flavors is, however, not inhibited.

The growth of coliforms can be prevented by using a fast-souring starter that rapidly converts lactose, thereby decreasing the pH in a short time to a level

TABLE 24.1 Microorganisms that Can Cause Defects in Ripened Cheese Without a Surface Flora

Microorganism	Important source of contamination	Carbon source	Salt-sensitive	Killed by low pasteurization	Off-flavors	Gas in cheese	Remarks
Yeasts	Sour whey	Lactose	Mostly	Yes	Yeasty, fruity	(CO_2)	Sometimes in raw milk cheese
Coliforms	Milk, curd, whey	Lactose (citrate)	Yes	Yes	Yeasty, unclean	(H_2), CO_2	Early blowing[b]
Propionibacterium spp.	Dung → milk	Lactate	Yes	Yes	Sweet	CO_2	[c]
Lactobacillus casei, plantarum, brevis	Milk, curd	Amino acids (citrate)	Little	Yes	Unclean, sharp	CO_2	Widespread
Salt-tolerant lactobacilli	(Rennet), weak cheese brine	Amino acids	No	Yes	Phenol, putrid	CO_2	Form cracks
Lactococcus lactis var. *maltigenes*	Milk, starter, sour whey	Lactose	Yes	Yes	Burnt, malty	—	
Streptococcus faecalis var. *malodoratus*	Dung → milk, curd	Lactose	Little	Partly	H_2S	—	
Streptococcus thermophilus	Heating equipment	Lactose	Little	No	Unclean, yeasty	CO_2	
Clostridium tyrobutyricum	Silage → dung → milk	Lactate	Yes	No	Sharp, putrid	H_2, CO_2	
Yeasts[a]	Brine, cheese shelf	Lactose, lactate	Variable	Yes	?		Slimy rind
Coryneforms[a]	Cheese shelf	Lactose, lactate	Variable	Yes	(Cabbage)		Slimy, red rind
Aspergillus versicolor, etc.[a]	Air, cheese shelf	E.g., lactose	Little	Yes	Musty		Produces sterigmatocystine

[a] Grow on the surface (aerobically).
[b] Except coliforms, all bacteria producing gas in cheese can cause late blowing.
[c] Desirable in some cheese varieties.

that inhibits growth. Moreover, after sufficient souring, the cheese should be low-ered in temperature and be salted as soon as possible.

The development of texture defects by coliform bacteria can be counter-acted by adding a sufficient amount of an oxidizing salt to the cheese milk, i.e., sodium or potassium nitrate (saltpeter). The nitrate suppresses the formation of the enzyme system normally involved in the production of H_2 (lactose $\rightarrow$ formic acid $\rightarrow$ H_2, under sufficiently anaerobic conditions) and induces the formation of nitrate- and nitrite-reducing enzyme systems. In effect, nitrate and nitrite act as hydrogen ion acceptors and no H_2 is produced from formic acid. The nitrogen of nitrate and nitrite is used for the bacterial growth or for am-monia production. The growth of coliform bacteria is not prevented by nitrate, the production of CO_2 is not affected, and the development of off-flavors is not inhibited.

Fermentation by coliforms has little effect on the pH of the cheese.

24.2 BUTYRIC ACID BACTERIA

In cheese certain anaerobic spore-forming butyric acid bacteria can grow and ferment lactic acid; the pH of the cheese is increased by the fermentation. The main breakdown products are butyric acid, CO_2, and H_2:

$$2\ CH_3 \cdot CHOH \cdot CO_2H \rightarrow CH_3 \cdot CH_2 \cdot CH_2 \cdot CO_2H + 2\ CO_2 + 2\ H_2$$

As a result, butyric acid fermentation leads to texture and flavor defects. In serious cases, cracks or large spherical holes are formed in the cheese as are very bad off-flavors. Because of this, growth of butyric acid bacteria in cheese is a serious defect. The butyric acid blowing manifests itself after several weeks, or even months, and is thus an example of late blowing. *Clostridium tyrobutyricum* is the main agent causing the defect; unlike other lactate-fermenting clostridia, it does not decompose lactose. *C. butyricum* can also cause defects.

Fermentation of butyric acid bacteria depends on:

a. The *number of spores* of butyric acid bacteria present in the cheese milk and their virulence after germination. Silage of poor quality is the main source of contamination because it contains large numbers of spores, which survive the passage through the digestive tract of the cow and are accumulated in the manure. The number is further deter-mined by the hygienic standards during milking. Even with modern milking methods a slight contamination of the milk by spores via ma-nure particles on the udder surface cannot be prevented. The spores survive pasteurization of the cheese milk. Their number can be reduced to a small percentage by bactofugation of the milk. Rigorous hygienic standards at milk collection are, however, paramount, at least for the

    manufacture of types of cheese in which a very low number of spores in the milk (some 5–10 per liter) can cause butyric acid blowing (see point d, below).

b. *pH and undissociated lactic acid content of the cheese*. A high level of lactic acid has a growth-inhibiting effect; at a lower pH, a smaller fraction of the lactic acid is dissociated. It is uncertain as to what extent the pH as such is of importance. At a very low pH (e.g., 4.6), butyric acid fermentation does not occur.

c. *The NaCl content of the cheese moisture*. Its growth-inhibiting effect closely depends on the pH (or the lactic acid content). The higher the pH, the higher the salt content must be to avoid growth of *C. tyrobutyricum*. The rate at which growth-inhibiting salt levels are achieved in the cheese is also crucial. For example, butyric acid fermentation is hardly a problem in Cheddar cheese (initial pH mostly 5–5.2) where the salt is mixed with the curd. Much greater difficulties are encountered with cheese in which the salt becomes slowly distributed throughout the loaf, i.e., large cheeses that are brined.

d. *Addition of nitrate*. Since about 1830, nitrate has been added to cheese milk to control butyric acid fermentation in cheese, especially in those varieties that have a favorable initial pH for the fermentation and are subject to a slow salt absorption process as occurs in Gouda-type cheeses. Nitrate as such is ineffective. The inhibition mechanism requires the presence of the enzyme xanthine oxidase (EC 1.1.3.22), which reduces nitrate to nitrite. Nitrite, or one of its degradation products, prevents the germination of the bacterial spores, be it only for a limited period. After that, the inhibitory action should have been taken over by a sufficiently high salt-in-water content.

    Traditionally, nitrate was added to the milk. Presently, it is often added to the mixture of curd and whey after most of the whey has been removed. Other conditions being equal, the required amount of nitrate depends on the number of spores of butyric acid bacteria in the milk. The critical concentration of spores is defined as the minimum number of spores per milliliter of milk (containing a certain amount of nitrate), capable of causing butyric acid blowing in the cheese made of that milk. For the purpose of illustration, the critical concentrations for the production of a 12-kg Gouda cheese, made of milk with 15 and 2.5 g added nitrate per 100 kg, are 20 and 0.25–1 spores per ml of milk, respectively. In Emmentaler and related cheeses, which have a fairly high initial pH and in which salt penetrates only very slowly, the critical spore concentration is nearly zero.

    Addition of more nitrate causes a higher nitrate level in the maturing cheese. During maturation, the concentration keeps decreasing, more

rapidly if the initial concentration was higher, but a certain amount is left. A higher level of nitrate only temporarily raises the nitrite concentration. The latter remains low; in Gouda cheese the maximum detectable amount of nitrite was found at only a small percentage of the amount of nitrate lost (Fig. 24.1). The causes of the losses of nitrate and nitrite are not well known.

Butyric acid blowing in cheese is determined by interaction of the various factors mentioned: the number of spores in the milk, the pH, the initial nitrate content of the cheese, the salt-in-moisture content, and the rate at which the salt becomes distributed throughout the cheese. Also, the ripening temperature has an effect (see point f, below). The release of any of these constraints, even temporarily, may initiate the growth of *C. tyrobutyricum*. Once the fermentation gets started, it continues at an ever-increasing rate, since it causes the pH to rise, which increasingly favors the conditions for growth.

A high number of coliforms may promote butyric acid fermentation because they rapidly consume the nitrate. Growth of mesophilic nitrate-reducing lactobacilli may have the same effect. Cheese made from milk heated to such an extent that xanthine oxidase is inactivated (see Fig. 22.1) is very susceptible to fermentation.
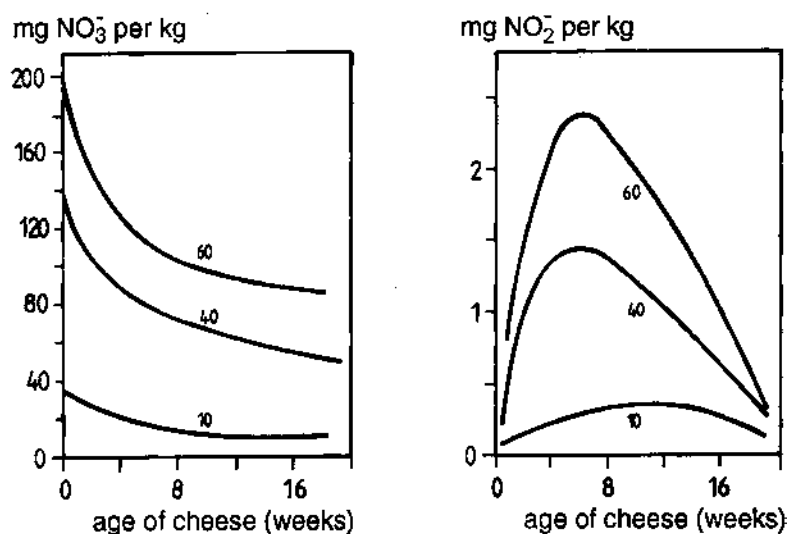
FIGURE 24.1   Contents of nitrate and nitrite of Gouda cheese during storage at 13°C. Parameter is the amount of $NaNO_3$ added to the cheese milk (g/100 L). Approximate results after Goodhead et al., *Neth. Milk Dairy J.* **30** (1976)207.

Sometimes traces of nitrosamines are found in cheese (e.g., 0.2 μg/kg), but the levels are neither related to the amount of nitrate in the cheese nor to its degradation. Moreover, reducing the amount of added nitrate to the milk does not significantly diminish the human intake of nitrate. For example, a daily consumption of 30 g nitrate-containing cheese contributes to less than 1% of the daily total intake of nitrate and nitrite. Nevertheless, in some countries increasingly lower amounts of nitrate in cheese are tolerated and, consequently, alternative methods are frequently tested to prevent butyric acid fermentation. The following methods are mainly applied:

1. *Bactofugation* of the cheese milk. A certain amount of nitrate remains necessary, but it can be considerably reduced, e.g., from 15 to 2.5 g nitrate per 100 kg of milk.

2. Addition of *lysozyme*. Usually, 2.5 g lysozyme per 100 L of cheese milk, corresponding to 500 enzyme units per ml of milk (250–300 mg per kg of cheese), is added; it is harmless to human health. Due to its association with casein micelles, lysozyme is almost quantitatively retained in the cheese. The action of the enzyme is based on the rupture of the peptidoglycane bonds in the bacterial cell wall, causing lysis. In the case of *C. tyrobutyricum*, lysis has already begun in the germinating spore. Lysozyme is not active at high NaCl content (e.g., 5% in water).

    Usually, gram-positive bacteria are much more susceptible to lysozyme than gram-negative ones. At moderate concentrations of the enzyme lactic acid bacteria are, however, little or not affected. Propionic acid bacteria are also impervious; hence, lysozyme can be used in Emmentaler cheese manufacture (see also point f, below).

    The usual dosage of lysozyme does not suffice for every cheese variety to prevent butyric acid fermentation because some strains of butyric acid bacteria (presumably *C. butyricum*) are not very sensitive to lysozyme. Simultaneous addition of some nitrate may thus be necessary.

3. In some cases, *formaldehyde* is added. Formaldehyde is a powerful inhibitor of butyric acid fermentation, but its use is illegal in most countries.

e. *Ripening time*. In cheeses with a short ripening time, the defects occur less frequently if at all.

f. *Ripening conditions*. A lower temperature causes a decrease in the growth rate of *C. tyrobutyricum* (minimum growth temperature is 7°C). In practice it is not possible to select a very low ripening temperature because of its influence on the maturation process. The high ripening

temperature of Emmentaler cheese, together with a high pH and a low salt content, make this type of cheese very susceptible to butyric acid fermentation.

24.3 LACTOBACILLI

Growth of mesophilic lactobacilli may induce texture and flavor defects. The organisms involved may be classified into common (*L. plantarum, L. casei, L. brevis*) and salt-tolerant lactobacilli. Salt-tolerant lactobacilli are related to *L. casei* and *L. plantarum* (but differ as to their salt resistance) as well as to *Bacterium proteolyticum*.

24.3.1 Common Lactobacilli

Even if initially present in only small numbers, say, 10 per ml of cheese milk, some strains may grow in cheese to reach a count over 2×10^7 per gram in 4–6 weeks, causing various gassy and putrid flavors and, often, excessive openness. Presumably, amino acids are used as a carbon source. In raw milk cheese, growth can hardly be prevented. The organisms are killed by low pasteurization of the milk. In cheese making, continuously operating curd drainage equipment can be an important source of contamination. Incidentally, some strains of lactobacilli are considered to cause the formation of desirable flavors in some cheese, e.g., Cheddar.

In Cheddar and Parmesan cheese species of *Pediococcus* closely related to these lactobacilli are also found; they cause similar defects.

24.3.2 Salt-Tolerant Lactobacilli

Salt-tolerant lactobacilli are a main cause of concern if cheese is salted in brine of reduced strength. Some strains can survive even in the presence of over 15% NaCl ($a_w \approx 0.89$). Apart from their salt resistance, they also differ from normal lactobacilli in their highly active amino acid metabolism, sometimes resulting in an excessive production of CO_2. Their growth causes texture defects and phenolic, putrid, mealy, fruity, or H_2S-like flavors in 4- to 6-month-old cheeses. A count over 10^3 of gas-forming, salt-tolerant lactobacilli per milliliter of brine is considered dangerous. The bacteria enter the cheese during brining. Penetration is facilitated if the cheese has been unsatisfactorily pressed, causing the rind to be insufficiently closed. The organisms usually do not grow in brine, not even in weak brine, and after they have contaminated the brine they die gradually. Lactobacilli can grow in deposits on the walls of basins and cages just above the brine level. Growth conditions for the lactobacilli are more favorable in these deposits due to an increased pH as a result of the growth of salt-tolerant yeasts, a lower NaCl content due to absorption of water, and a slightly higher tempera-

ture than that of the brine. Measures to keep the count of lactobacilli low include satisfactory hygienic measures in the brining room (e.g., removal of deposits), maintaining a NaCl content of the brine of at least 16% and a pH <4.5. Obviously, contamination of the cheese milk by these bacteria should be avoided.

24.4   HEAT-RESISTANT STREPTOCOCCI

Especially strains of *S. thermophilus* can cause defects. Contrary to mesophilic streptococci, they grow at 45°C and survive thermalization and low pasteurization of milk. During these heat treatments they may become attached to the wall of the cooling section in the heat exchanger and multiply very rapidly (minimum generation time about 15 min), partly depending on the initial number in the raw milk. Continuous use of heat exchangers for a long time without intervening cleaning may heavily contaminate the cheese milk, to reach a count up to 10^6 bacteria/ml. Concentration in the curd and growth during the early stages of cheese making may increase the count to over 10^8 per gram of cheese. Unclean and yeasty flavors develop. Moreover, CO_2 production by the bacteria may yield cheese with excessive openness, especially when a starter with a high CO_2-producing capacity is used.

24.5   PROPIONIC ACID BACTERIA

Growth of propionic acid bacteria is desirable in certain varieties of cheese (e.g., Emmentaler cheese; see Section 25.4) to achieve a satisfactory quality; the bacteria are added to the cheese milk. In other cheeses, excessive growth of these bacteria causes defects (Table 24.1). The bacteria are killed by low pasteurization of the milk. Obviously, they are predominantly of interest in the manufacture of raw milk cheese.

The most important species is *Propionibacterium freudenreichii* var. *shermanii*. Most of the species ferment lactose, and all ferment lactic acid. In cheese the lactose fermentation goes unnoticed because the bacteria multiply slowly and cannot compete with the starter bacteria. Lactic acid is converted to propionic acid, acetic acid, CO_2, and water, according to the general formula:

$$3\ CH_3 \cdot CHOH \cdot CO_2H \rightarrow 2\ CH_3 \cdot CH_2 \cdot CO_2H + CH_3 \cdot CO_2H + CO_2 + H_2O$$
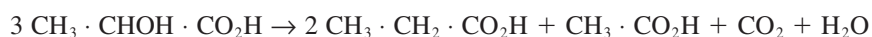
Clearly, the pH of the cheese is little affected by fermentation.

A distinct propionic acid fermentation results in excessive gas formation and the development of a sweet taste. In Gouda-type cheese such fermentation is considered a defect. Since the bacteria grow very slowly in cheese, any serious defects appear only after a prolonged ripening time and are a form of late blowing.

Conditions determining the growth of propionic acid bacteria in cheese are as follows:

a. *Acidity*. The organisms grow little or not at all at pH ≤5.0. The growth rate increases with increasing pH.

b. *NaCl content* in the cheese moisture. Increasing the concentration retards the growth of the bacteria; the NaCl content in most cheeses (<5% NaCl in the water) is too small to be effective, but inhibitory concentrations can occur in the rind of cheese shortly after brining.

c. *Storage temperature*. Increasing the temperature favors the growth. (In the manufacture of Emmentaler cheese advantage is taken of this effect, to enhance the propionic acid fermentation; see Section 25.4).

d. *Presence of nitrate*. The fermentation is slowed down, probably due to the formation of nitrite.

When conditions allow growth of propionic acid bacteria in cheese, butyric acid bacteria, if present, may also be expected to develop.

24.6   ORGANISMS ON THE RIND

Abundant growth of yeasts and coryneform bacteria on the cheese surface may cause a slimy rind and a parti-colored or pinkish appearance. Growth of these organisms is enhanced by inadequate drying of the rind after brining, and also by a significant lactose content in the rind due to insufficient souring of the cheese, by salting of cheese in weak brine of a high pH, and by use of poorly cleaned shelves in the curing room. Growth of molds causes discoloration and a musty flavor; under extreme conditions it may produce a health hazard because of mycotoxin formation. It particularly involves *Aspergillus versicolor*, which, under some conditions, can produce sterigmatocystine (see Section 23.8).

To prevent growth of the organisms involved, special attention must be paid to the treatment of the cheese rind and to the hygiene and air conditioning in the curing room (see Section 22.5).

24.7   SOME OTHER MICROBIAL DEFECTS

Growth of certain lactobacilli (e.g., *L. büchneri*) and/or of fecal streptococci may cause undesirable high levels of amines (especially histamine and tyramine), usually in cheese made of raw milk (see Section 23.8).

Several microorganisms can cause flavor defects, predominantly in raw milk cheese. Among them are yeasts (yeasty, fruity flavor), *Lactococcus lactis* var. *maltigenes* (burnt flavor) and *Streptococcus faecalis* var. *malodoratus* (H_2S-like, gassy, unclean flavors); if present in increased numbers these enterococci

can also cause texture defects due to the production of CO_2 from amino acids. Many organisms can cause bitterness in cheese.

Increased levels of psychrotrophs or of their thermostable lipases in the cheese milk may cause the cheese to turn rancid.

24.8 ESTABLISHING TYPES OF MICROBIAL DEFECTS WITH GAS PRODUCTION

If gas formation is excessive, the smell and taste of the cheese may already be indicative of the type of fermentation involved. To establish the fermentation in cheese with less serious defects, determination of the redox potential (E_h) is essential. In cheese of pH $\sim$5.2, in which no gas besides some CO_2 is formed, the E_h amounts to -140 to -150 mV, as measured with a normal hydrogen electrode. In cheese with H_2 formation and of the same pH, the E_h drops to -250 to -300 mV. If a more detailed classification is desired, microbiological assays can be performed (including growth in selective culture media, microscopic examination of the bacteria involved) as well as a determination of the fatty acid content of the cheese (butyric acid, propionic acid).

SUGGESTED LITERATURE

- Microbial defects are for most part discussed for specific cheeses (see references in Chapter 25). A general overview of health hazards caused by bacteria is:

    E. A. Zottola and L. B. Smith, in: P. F. Fox, ed., *Cheese: Chemistry, Physics and Microbiology, Vol. 1, General Aspects*, 2nd ed., Chapman and Hall, London, 1993, Chapter 12 (Growth and survival of undesirable bacteria in cheese).

25

Cheese Varieties

Cheese can vary greatly in composition and is manufactured and cured in widely varying manners. This results in a bewildering variety of types. In this chapter the variability as such is discussed first: What can be varied and what are the consequences? Subsequently, several cheese types are discussed in more detail. This illustrates the interaction between the various process steps and the need to integrate the steps into an efficient process, leading to a good-quality cheese of the type desired. Of course, a selection among the numerous types produced had to be made. The main consideration for selection has been that the most important aspects of cheese making and cheese properties are treated. Moreover, the economic importance of some cheese varieties has been taken into account.

25.1 OVERVIEW

The word ''cheese'' is commonly used as a collective term for widely variable products such as matured and nonmatured cheese made with rennet, acid curd cheese, fresh cheese, and even processed cheese. Most of these fit the definition formulated by the FAO/WHO[1]:

> Cheese is the fresh or matured solid or semi-solid product obtained by coagulating milk, skimmed milk, partly skimmed milk, cream, whey cream, or buttermilk, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from such coagulation.

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[1] FAO/WHO Standard No. A-6 (1978).

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The drainage of whey is an essential aspect of the definition. Concentrated products, obtained by removal of water only, are considered milk products. Also the Norwegian ''mysost'' (whey cheese) is not a cheese according to the definition. It is made by evaporating whey or a mixture of whey and milk or cream until a brown mass forms that coagulates after cooling. The main constituent is finely crystallized lactose.

Furthermore, cheese can be modified afterward, e.g., to obtain processed cheese. In some countries, these and other preserves of cheese are not allowed to be referred to as cheese per se. They are briefly reviewed in Section 25.7.

Throughout the ages numerous varieties of cheese have evolved. The development of a cheese closely depended on local conditions, including the amount and type of milk available, climate, presence of suitable caves for storage, the need for a long shelf life (compare, for instance, milk production on elevated mountain meadows with that in densely populated regions) and other economical and geographic factors. Furthermore, it was a matter of accumulated technological experience and of finding, by trial and error, the manufacturing conditions that led to a product being tasty, easy to handle, and durable. Along with this, the general level of technological and hygienic know-how were of great importance. Various process steps that now are very common are relatively new, say 100 years old: use of starter, use of industrially made rennet, washing of the curd, pasteurization of the cheese milk. Of more recent date are the use of inocula for the surface flora, application of latex emulsions to the cheese rind, use of enzyme preparations to accelerate ripening, and so forth.

Throughout the ages several types of cheese greatly changed in character. They usually kept their names, e.g., because the name was that of a region or of its principal market town. Furthermore, when a variety of cheese was well reputed, other individuals would see advantage in borrowing its name. Often, technological know-how was ''exported.'' At present the names of some varieties have been protected internationally, but most are not.

25.1.1 Variations in Manufacture

Following are the main variations applied in the manufacturing process:

   a. *The kind of milk*. Milk of cows, goats, sheep (ewes), and buffaloes differ in composition. The fatty acid composition of the milk fat of the various animal species varies, which affects the flavor of the cheese. The short chain fatty acids C_6–C_{10} cause a sharp flavor in goats' milk cheese. A similar flavor may form in ewes' milk cheese, but only if growth of molds, etc., occurs (as in Roquefort cheese), since ewes' milk has very little lipase activity. Cheese made from sheep, goat, or buffalo milk has a fairly white color because those milks contain hardly any carotene (they do contain vitamin A). Other

TABLE 25.1 Compositional Data of Milk of Certain Animal Species, of Importance for the Cheese Made of the Milk Involved[a]

Animal species	Casein (%)	Fat/casein	Lactose[b] (%)	Casein composition[c]				
				α_s	:	β	:	κ
Cow	2.6	1.5	4.9	49		38		13
Goat	2.6	1.7	4.6	25		55		20
Sheep	3.9	1.8	5.4	54		34		12
Buffalo	3.2	2.3	5.4	47		40		13

[a] Approximate averages.
[b] In the fat-free and casein-free milk.
[c] Quite variable, especially in the goat.

differences are delineated in Table 25.1. They affect the cheese yield, the fat content, and the pH. Probably, the low level of α_{s1}-casein in goats' milk (varying from zero to 20% of casein) is responsible for the short texture of goats' milk cheese. Curd of goats' milk synereses more strongly than that of cows' milk.

b. *Standardization of the milk*. An important characteristic of cheese is its percentage of fat in the dry matter. This content is closely connected with the percentages in the milk. Changing the ratio between fat and fat-free dry matter in the cheese milk affects clotting and syneresis, and hence water content and pH of the cheese. Flavor and consistency are linked with the fat content in the dry matter. Fresh cheese is often made of skim milk. In ripened cheese the fat content in the dry matter ranges from 20% to 60%. Full-fat cheese, i.e., cheese made from whole milk, usually contains from 46% to 52% fat in the dry matter, primarily depending on milk composition.

c. *Heat treatment of the cheese milk* (see also Fig. 22.1). The heating intensity affects the type and extent of the bacterial flora of the milk (and hence the need to use a starter), the activity of lipase, the rennetability of the milk, the tendency to synerese, and the retention of serum proteins in the cheese. In the manufacture of some varieties of fresh cheese the milk is high-heated during coagulation with acid.

d. *Preacidification of the cheese milk*. Preculturing or ripening the milk during some 1–3 h (previously sometimes much longer) prior to rennet addition affects clotting and syneresis. In addition, preacidification has a direct effect on the pH of the cheese because a greater amount of colloidal phosphate already dissolves during curd manufacture; much of the dissolved calcium and phosphate are lost with the whey,

thereby diminishing the buffering capacity of the cheese as well as the yield.

e. *Starter and inoculum percentage* (0% to 5%). Processing conditions, especially the desired rate of acid production and the scalding temperature, as well as the processes desired during maturation, determine what species and strains of lactic acid bacteria are selected. Considerable variation exists among species, often resulting in cheeses of different type with respect to gas production, flavor, etc. Even strains of one species differ considerably in rate of acid production, in rate and ''depth'' of proteolysis, and in production of desirable and undesirable flavor compounds. Low inoculum percentages or use of less active strains are responsible for a slow acid production in the cheese. Such a slow production is necessary for certain soft cheeses but is undesirable for most cheeses, e.g., Gouda types, because of the increased risk of defects, and especially Cheddar types, because of the increased time needed until salting.

f. *Addition of a secondary microflora*. In the manufacture of many types of cheese, in addition to the starter other microorganisms are added to the milk or to the cheese rind. All cheeses with large ''eyes'' (Emmentaler, Gruyère, Jarlsberg) contain propionic acid bacteria in addition to lactic acid bacteria. For a desirable surface flora to develop, the cheeses or the milk are exposed to bacteria (coryneform bacteria) or molds (*Penicillium*). In blue-veined cheese internal mold growth is further stimulated by piercing air channels in the loaves.

Generally, the added microorganisms are mesophilic or thermophilic, and decompose lactic acid. Their growth has a considerable effect on the pH, hence on consistency and flavor. Furthermore, most of them cause proteolysis and lipolysis and can form flavor compounds.

g. *Way of coagulation and type of coagulant*. Coagulation of the milk can occur by means of acid, rennet, or a combination of renneting enzymes and acid. The acid can originate from in situ production of lactic acid as well as from direct acidification with added acid. Besides calf rennet, other renneting enzymes are applied, including pepsin from cow, pig, or chicken, vegetable enzymes (e.g., ficin, papain, bromelin), and enzymes from molds (e.g., *Mucor pusillus, Mucor miehei*) or from bacteria. Composition and structure of the various milk gels differ, hence syneresis, curd fusion, etc. Amount, activity, and character of the renneting enzymes retained in the cheese greatly affect the ripening.

h. *Curd making*. The rate and extent of syneresis greatly depend on the size of the curd grains and on the intensity and duration (0–5 h) of

stirring. The treatment of the curd is among the important process variables to regulate the moisture content of the cheese. The stirring intensity also increases with the amount of whey removed.

i. *Scalding (cooking) temperature* (see Fig. 25.1). Stirring the curd at a higher temperature increases the rate of syneresis. The scalding temperature also affects the amount of rennet in the curd. If the temperature is higher, less rennet is retained in the cheese and the proteolysis rate during the maturation is slower. Besides, at still higher temperatures, i.e., higher than 50°C, rennet is inactivated, at least at not too low pH as, for instance, in the manufacture of Emmentaler. Complete inactivation of the enzyme in stretched curd cheese has not yet been established; to be sure, in the manufacture of this cheese the temperature becomes high, but the pH is much lower and at lower pH chymosin is much stabler. Likewise, the activity of the starter bacteria
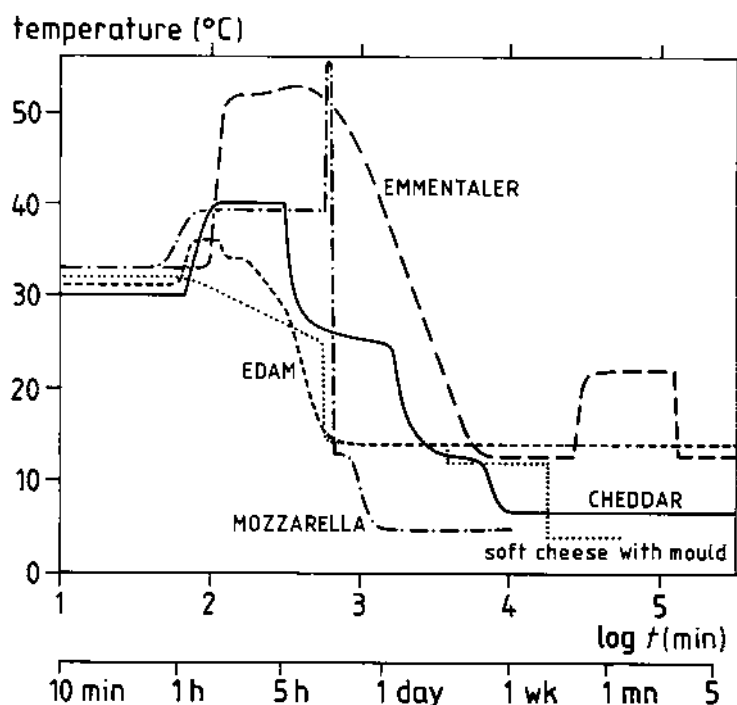
FIGURE 25.1 Course of the temperature during manufacture and ripening of some types of cheese; t = time after renneting. Approximate examples.

depends on the scalding temperature and duration. The activity of most mesophilic strains decreases at temperatures above, say, 30°C.

j.   *Washing of the curd*. Addition of water (up to 200%) decreases the concentration of lactose in the moisture of the cheese, hence the ratio between lactic acid and buffering compounds. This ratio determines the pH of the cheese. The larger the amount of whey drawn off and the larger the amount of water added, the more effective the washing.

k.   *Size and shape of the cheese*. Shape and weight are among the factors defining the variety of the cheese. However, these factors do not only refer to the appearance of the cheese; they are linked with the desired type since the specific surface area (which ranges from 1 to 20 dm^2/kg) greatly affects several changes during manufacture and curing. The smaller and flatter the loaf, the faster the cooling (during pressing, resting, brining, etc.); the faster the salt absorption during salting; the greater the effect of any surface flora on the ripening; the faster evaporation of water during storage. Large cheeses require relatively less labor and maintenance during curing. A large cheese must be firm, i.e., have a high viscosity, because otherwise it will sag too much.

l.   *Pressing*. Pressure is mainly applied to form a closed rind, hence to extend the shelf life. Small cheeses with surface ripening are commonly not or only lightly pressed. Poorly fusing curd, such as strongly acidified curd for Cheddar cheese manufacture, is often pressed under vacuum to obtain a more closed texture.

m. *Resting*. The period between pressing and salting (ranging from 1 to 24 h), or that between pitching and salting for Cheddar types (see variable *n*), is variable and mainly depends on the rate of acid production. Most of the lactose should have been converted to lactic acid before the lactic acid bacteria are slowed down by the salt. A rapid acid production is needed to prevent growth of undesirable bacteria (e.g., coliforms) and to speed up manufacture.

n.   *Method of salting*. Salting the cheese after pressing and resting fundamentally differs from salting by mixing salt with soured curd before pressing, as is done in the manufacture of Cheddar and Cantal. Dry salting or rubbing the cheese with brine is applied if a surface flora of yeasts and bacteria should develop.

o.   *Salt content* can vary widely, from 0.5% to 10% for ripened cheeses. White pickled cheese, a well-known example being feta, is stored in brine. Cheese made of milk of poor bacterial quality needs a high salt content to attain a certain shelf life. The flavor, consistency, and ripening of the cheese (especially proteolysis and lipolysis) are markedly affected by the salt content.

p. *Additives*. Spiced cheeses are made of old. They are made chiefly with cumin or with caraway. The spices are usually mixed into the curd after the whey has been drawn off. The range of additives has increased in recent years, e.g., with cheeses containing chives, green pepper, nettles, parsley.

q. *Ripening temperature* is generally between 5°C and 20°C. The higher it is, the faster all kinds of ripening processes occur. Lipolysis is more dependent on temperature than is proteolysis. As a rule, a high temperature eventually leads to a less satisfactory flavor, but that depends greatly on other factors. Gas production, e.g., by propionic acid bacteria, proceeds much more quickly at a higher temperature. Because of this, such cheeses as Emmentaler are kept for a certain time at a higher temperature (see also Fig. 25.1). At high temperature the cheese can readily sag, oil off, evaporate water at low air humidity, and show early microbial defects (Chapters 23 and 24).

r. *Handling during storage*. This concerns the treatment of the cheese during curing, especially the treatment of the rind. If the rind must remain clean, the cheese should be rubbed off regularly and the relative air humidity (RH) kept low, i.e., 80% to 85%, unless microbial growth is hindered otherwise. A very low RH causes marked evaporation of water and cracks in the rind. If a white mold should be present on the rind its growth should be enhanced by leaving the cheese on mats or gauze and by keeping the RH at a higher level, i.e., 85% to 90%. To have blue mold growth, holes should be pierced in the cheese. For a red smear the RH should be still higher, i.e., 90% to 95%; the air may even be nearly saturated with water vapor, and the cheese rind should be rubbed regularly with salt or brine and the cheeses be turned. The duration of the different treatments may vary.

s. *Ripening time* generally ranges from 1 day to 3 years. Usually, the eating quality of a cheese (flavor and consistency) increases after a given ripening time and decreases again subsequently. In some varieties this decrease occurs rapidly, in others slowly. Generally speaking, the earlier the optimum occurs, the shorter it lasts (Fig. 23.10). As a rule, the ripening phase is a not quite independent variable because it should depend on composition, size, and ripening conditions of the cheese. For many hard and semihard cheese varieties, such as Gouda and Cheddar types, the ripening time can be varied widely. Anyhow, to achieve a satisfactory eating quality the composition of the cheese, especially the moisture content, should be adapted (see also Table 22.3 for variables *q*, *r*, and *s*).

t. *Covering the cheese rind and packing*. Covering the surface with a latex emulsion can prevent, or at least decelerate, growth of microor-

ganisms on the rind. A red smear can even be dried up and subsequently be protected with a latex emulsion. Furthermore, covering of the rind (especially with paraffin) and packing of the cheese (wrapping in Saran or other plastic foils) are specifically meant to prevent water evaporation. A ripening cheese that forms NH_3 or other gases should be packed in such a way that the gases can escape.

u. *Several modern techniques*, such as continuous renneting, ultrafiltration, bactofugation, homogenization, and addition of enzyme preparations, have not yet resulted in varieties that differ materially from the existing ones. However, they may greatly affect some properties of the cheese.

The sum of all different combinations of variables would lead to an impossibly high number of cheese types, say 10^{16}, but that is not realistic. Many combinations make no sense, a great many achieve a poor result, whereas still others cause no appreciable differences. We will therefore try to introduce some order in the variability by considering the following main variables in the manufacture of cheese:

1. *Adjustment of the moisture content*, i.e., essentially the ratio between water and protein (w/p). The moisture content is affected by many process steps. In effect the moisture content also roughly determines the size of the cheese (see Fig. 25.2), since a large cheese inevitably is somewhat dry (Section 22.3). In theory, a small cheese can be made dry, but it would soon become too hard because of water evaporation. The most important point probably is that the moisture content determines the shelf life. Fresh cheeses ($w/p \approx 4$ or higher) become up to 1 week old, soft cheeses ($w/p \approx 2.5$), e.g., 1 month, semihard cheeses ($w/p \approx 1.5$–2) up to several months, and hard cheeses ($w/p < 1.2$) several months up to several years (e.g., Parmesan cheese).

2. *Adjustment of the pH* (see also Fig. 25.3). Unless a very dry cheese is made the pH would generally become too low. This effect can be counteracted, however, by several methods. The curd can be washed as is applied for Gouda-type cheese. Furthermore, the acid production can remain slow by applying little or no starter (and having milk of a low bacterial count) and it may be retarded further by a low temperature (cottage cheese, Butterkäse, Bel Paese) or by penetrating salt (Meshanger). Alternatively, lactic acid may be consumed by a surface flora (Camembert, Brie, Muenster), by internal molds (Roquefort, bleu d'Auvergne, Stilton), or by both (Gorgonzola). If the pH at the pressing stage is low, the cheese will have a low level of calcium (Fig. 25.4) and of phosphate.

log (*w/p*)
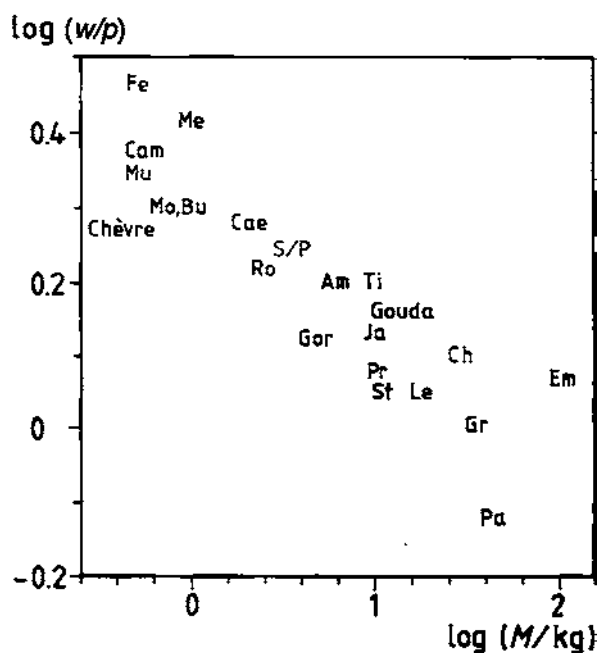
FIGURE 25.2 Approximate relationship between the water/protein ratio (*w/p*) and the mass (*M*) of most of the cheeses in Figure 25.5.

3. The *dominating enzymes*, largely originating from the cheese flora. This flora partly depends on the above factors (variables 1 and 2) and on the manufacturing temperature. It mainly concerns mesophilic or thermophilic lactic acid bacteria. In addition, propionic acid bacteria, white molds, blue molds, or a red smear flora of yeasts and bacteria may be present. The surface flora essentially is a complex and changing mixture of organisms. Besides microbial enzymes, the amount and type of the renneting enzyme retained in the cheese is important. The contribution of the various enzymes to proteolysis can vary widely among cheese varieties, as is shown in Table 23.1.

25.1.2 Types of Cheese

How can the different varieties best be classified? The technologist is inclined to take differences in the manufacturing process as the characteristics. Of course, perceptible properties of the cheese prevail for the consumer. These include size, shape, outer and inner appearance (''body''), and especially flavor and consis-
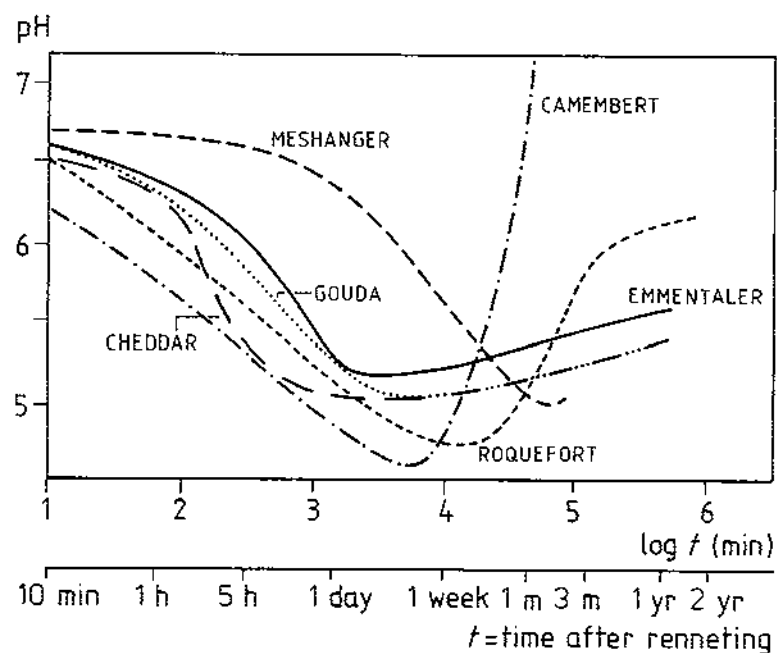
FIGURE 25.3 Course of the average pH during manufacture and ripening of some cheese varieties. Approximate examples.

tency. The latter two features mainly depend on the composition in its broadest sense, i.e., including pH, free amino acids, H_2S, etc. Some differences in texture, especially number and distribution of eyes, depend on the means of manufacture and hardly at all on composition; these differences are, however, of minor importance. Furthermore, Gouda cheese made by a cheddar process may be hard to distinguish from true Gouda, except for those eyes.

All in all, the main variables are the ratio between water and protein and the type of ripening, i.e., fresh or matured, type of starters, secondary organisms, ripening phase. So we arrive at Figure 25.5, which shows some important varieties as well as some widely variable types. Some features not mentioned are fat content in the dry matter, pH, salt content, and ripening time. As shown in Figure 25.2, size and moisture content may be highly correlated, as they are with duration of ripening.

How many cheese varieties do exist? The answer is arbitrary because it remains undefined as to how large a difference must be for different varieties to exist. Many different designations may be in use for what is essentially the same
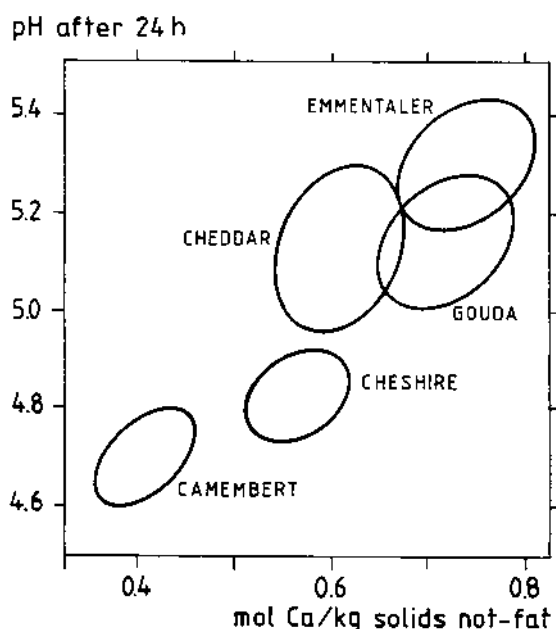
FIGURE 25.4 Approximate relationship between the pH after 1 day and the amount of calcium in the dry matter of some cheese varieties.

cheese variety, e.g., Gouda, Javor, Fynbo, Norvegia, Mazurski, prästost, kostromskoj syr. On the other hand, the denomination of Gouda may include a 6-week-old, somewhat soft cheese with little flavor, as well as a more than 1-year-old farmhouse cheese that is brittle and piquant; these two cheeses differ at least as much as do Saint Paulin and Gruyère. All in all, not more than a few dozen substantially different varieties can be distinguished. Most of these are shown in Figure 25.5.

Which cheeses constitute the (economically) most important varieties in the world? Most likely the following:

1. *Dutch-type varieties*, i.e., Gouda and Edam, including the numerous variants. These are defined as those that:

   Are made of cows' milk
   Have 40% to 50% fat in the dry matter
   Use mesophilic starters
   Are brine-salted after pressing
   Have a water content in the fat-free cheese below 63%

Starter		mesophilic		thermophilic		mesophilic		
Details		Fresh	Ripened	Propionic acid bacteria	—	Red smear	White mould	Blue mould
$\frac{w}{p}$ = 5	$\log \frac{w}{p}$	Quarg						
4	0.6	Cottage						
3			Feta[1,2]					
			Meshanger					
	0.4		Queso blanco[2,3]				Camembert	
2			Butterkäse		Mozzarella[5]	Munster	Lymeswold	
			Caerphilly[4]				Chèvre[2]	
			St. Paulin			Port Salut		Roquefort[2]
1.50	0.2		Amsterdammer			Tilsiter		
			Gouda					
			Jarlsberg		×——	——×——		Gorgonzola
1.25			Cheddar[4]	Emmentaler	Provolone[5]			
			Leyden					Stilton[4]
1	0			Gruyère ——		——×		
0.8					Parmigiano			

FIGURE 25.5 Classification of cheese varieties based on type of maturation and on moisture content, i.e., ratio of water to protein, w/p. 1, Kept in brine. 2, Milk may differ from cows' milk. 3, Usually by acid coagulation. 4, Salting of the curd prior to pressing. 5, Pasta filata. × means that the parameter involved applies as well.

Have no essential surface flora
Are matured from 2 to 15 months

2. *Cheddar-type cheeses*. These are much like the cheese of the previous group, but they are made in a somewhat different way, in that they are salted in the curd stage. They are on average slightly drier and slightly more acidic than the Dutch-type varieties and have a somewhat different flavor note.
3. *The group of fresh cheeses*, which have a high moisture content and are not or hardly matured.

Furthermore, fairly large quantities of the following cheeses are being produced:

Cheese with propionic acid bacteria (Emmentaler, Jarlsberg)
Stretched curd cheeses (provolone, kashkaval, mozzarella)

A semisoft type of cheese with a very mild flavor (Saint Paulin, Monterey, Amsterdammer)

White pickled cheeses (feta, Domiati)

Soft cheeses with a white mold (Brie and Camembert, often poorly resembling the original cheese that had a more distinct flavor)

Blue-veined cheeses (''bleu,'' Gorgonzola, Roquefort, Stilton).

Subsequent sections of this chapter deal with some varieties of cheese in more detail.

25.2 FRESH CHEESE

Fresh or unripened cheeses are curd-like products that can be consumed immediately after manufacture. The products undergo little, if any, ripening, except that lactose is fermented. Generally, fresh cheese has a limited shelf life, say, 2 weeks in the refrigerator.

Numerous varieties of fresh cheese exist and in many countries the quantities consumed are considerable. The cheeses may vary as to kind of milk, fat content, and means of manufacture. Often, the milk is coagulated by acid or by a combination of heat and acid. Sometimes a little rennet is used. Generally, the fresh cheese has a spreadable (i.e., soft and short) or even granular texture. A kind of archetype is bag cheese. In its manufacture sour milk or buttermilk is boiled, which (further) coagulates the casein. The mixture is suspended in a cheesecloth bag to allow leakage of whey. In the manufacture of the Latin American queso blanco, the milk is provided with acid (e.g., vinegar, lemon juice, HCl) and is heated with such intensity that most of the serum proteins are incorporated into the cheese. The Italian ricotta is made by heating a mixture of sour milk and sour whey. A different type of fresh cheese is renneted ''white cheese.'' Its manufacture often is similar to that of a small Gouda, except that the moisture content is high. It is lightly pressed and lightly brine-salted. The loaf is coherent..

It may be noted that there is a gradual transition from sour milk to ymer (see Section 20.2), to fresh acid cheese, to the white cheese just mentioned. Some typical examples of composition are given in Table 25.2.

In addition to traditional or domestic methods for manufacture, large-scale industrial processes have been developed that are often quite different. Preserves of fresh cheese are also made. Two often consumed products will be briefly considered.

25.2.1 Quarg

Quarg definitely has originated from bag cheese and sometimes it is still made in that way. Speisequark, Neufchâtel, tvorog, and baker's cheese are closely re-

TABLE 25.2 Approximate Composition of Fresh Cheese and Some
Comparable Products[a]

Product	Water (g)	Protein (g)	Fat (g)	Ca (mg)	P (mg)	Energy (kJ)
Concentrated yogurt	82	5	5	175	140	380
Ymer	85	6.5	3	150	160	300
Quarg, low-fat	83	12	0.2	120	180	270
Quarg, high-fat	74	10	12	120	180	660
Cottage cheese, creamed	78	12	4	100	180	430
''White cheese''	60	16	19	450	300	1030

[a] Quantities are per 100 g of product.

lated or identical products. Petit Suisse and Doppelrahmfrischkäse have an
increased fat content. Due to its neutral flavor and its consistency (smooth,
can be blended), quarg is a suitable ingredient in several dishes. At present,
low-fat quarg is usually made. Figure 25.6 gives an outline of a manufacturing
process.
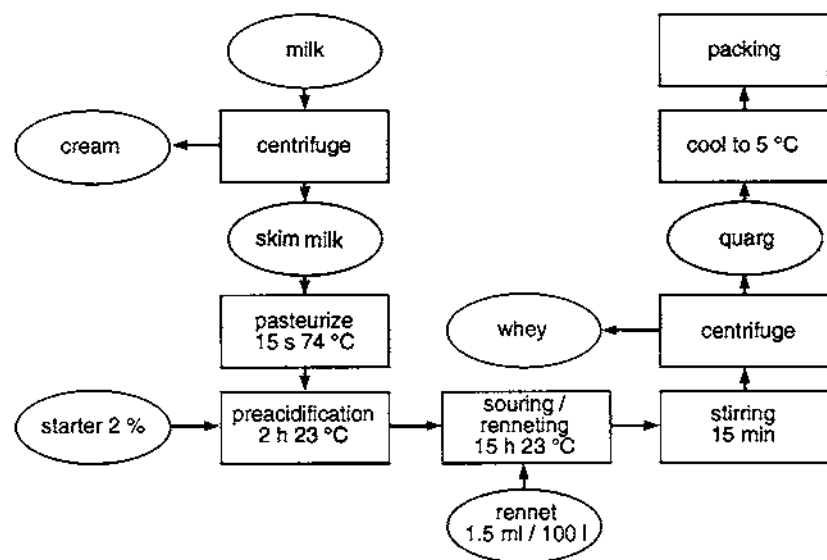
FIGURE 25.6 Example of the manufacture of low-fat quarg.

An aromatic starter is commonly used because of the flavor. The acid production may be done at a higher temperature to speed it up. Preculturing of the milk is not always applied. The breaking of the coagulum by stirring occurs when the pH has dropped to 4.6–4.7. At that pH the curd tends to synerese only slightly. Often a little rennet is added, causing the curd to become firmer and allowing the whey to be separated more readily. For the same reasons the milk is pasteurized at low temperature. Too high a level of rennet causes premature syneresis, hence an inhomogeneous product and formation of a bitter flavor during storage of the quarg.

Large-scale production units of quarg became feasible after introduction of the quarg separator. In this centrifuge, the clotted protein mass is separated from the whey by centrifugal force and discharged through small openings in the wall of the separator bowl. In this way a smooth product is obtained. The water content of the quarg can be satisfactorily adjusted by varying the flow rate. If the whey is separated in a different manner (usually by filtration), the quarg obtained should be smoothened (homogenized). Furthermore, centrifugal separation fails if quarg is made of whole milk because the difference in density is too small. Moreover, much of the fat is lost in the whey. Centrifugal force can be applied, however, by operating as follows. Diluted cream is homogenized at low temperature and high pressure. This causes the fat globules to be predominantly covered with micellar casein and to form homogenization clusters. After acidification, almost all of the fat globules participate in the protein network. Since the density of the protein and fat mixture involved is less than that of the whey, the product can be separated in a milk separator–type centrifuge. Then the desirable fat content of the final product can be adjusted by blending high-fat and low-fat quarg. All the same, it is much simpler to blend low-fat quarg with cream. This better masks the acid or astringent taste of low-fat quarg that many people consider unpleasant.

Alternatively, quarg is made by ultrafiltration of milk or skim milk. The retentate obtained is soured, cooled, and the coagulum broken by stirring. This results in a higher yield, since serum proteins and additional calcium phosphate are enclosed. However, the product has a different, less firm consistency. Furthermore, slightly more syneresis tends to occur during storage. Another method to increase the yield is to start from high-heated milk; sufficient whey can be separated by using the quarg separator. The product involved, again, is thin and somewhat sticky. Thickening agents may be added to arrive at a satisfactory consistency and to avoid wheying off during storage.

The shelf life of quarg is limited by proteolysis (bitter, cheesy) and by excessive acid production (sharp, acidic). In particular, contamination by yeasts and molds may reduce the keeping quality. Naturally, flavor deterioration and whey expulsion occur earlier when the product is kept at a higher temperature.

25.2.2 Cottage Cheese

Cottage cheese is an unripened, particulate, slightly acidic cheese made of skim milk. A little salt and fresh or cultured cream are generally added to the cheese. Distinctive features of the product are its granular form and, in spite of the low fat content, its creamy flavor.

The traditional manufacture of cottage cheese (Fig. 25.7) differs from that of quarg as to some process steps:

1. The clotted milk is cut into cubes of a desirable size and is not broken by stirring.
2. A non-gas-forming starter is applied. Otherwise the curd would start to float and it would be hard to drain off the whey.
3. Cooking (scalding) enhances syneresis. This is necessary because the pH is quite low and because the stirring should not be too intensive. Cooking also causes the curd to become firmer. To obtain a firm curd, the milk should be low-pasteurized and a little rennet is added.
4. The curd is thoroughly washed to remove most of the lactose and lactic acid. At the same time, the mixture is cooled.
5. The low-fat curd is blended with cream.

Usually, the starter is added to the milk together with the rennet. Sometimes the rennet is added $1-1\frac{1}{2}$ h after inoculation with starter. As in quarg manufacture, the process conditions can vary widely, e.g., from acid production at 32°C during 4–5 h with 5% to 6% starter added (short-set method), to acid production at 22°C for 12–16 h with 0.25% to 1% starter (long-set method).

The moment of cutting affects considerably the properties of the product. The firmness of the coagulum closely depends on the pH and, to a lesser extent, on the rennet action. Usually, acid is produced to pH 4.6–4.8. Curd cut at a lower pH remains weaker, whereas curd of a pH higher than 4.9 becomes too firm and tough.

The milk may also be acidified directly by adding inorganic or organic acids, i.e., the direct-set method. The acid is added at low temperature, say 7°C, while the milk is vigorously stirred until the pH is 4.6. Then, while the milk is at rest, its temperature is raised by ohmic heating (passing an electric current through the milk), which causes a uniform development of heat in the milk. Setting then takes about 12 min. Otherwise, the manufacture is as usual, but the flavor of the product is often considered to be less satisfactory.

Unlike the situation in pressed (mature) cheeses, the curd size and curd size distribution directly affect appearance and flavor perception of cottage cheese. The freshly cut curd is soft and fragile. After cutting, it is left for some 10–15 min to expel a little whey. Thereby the curd granules increase in firmness
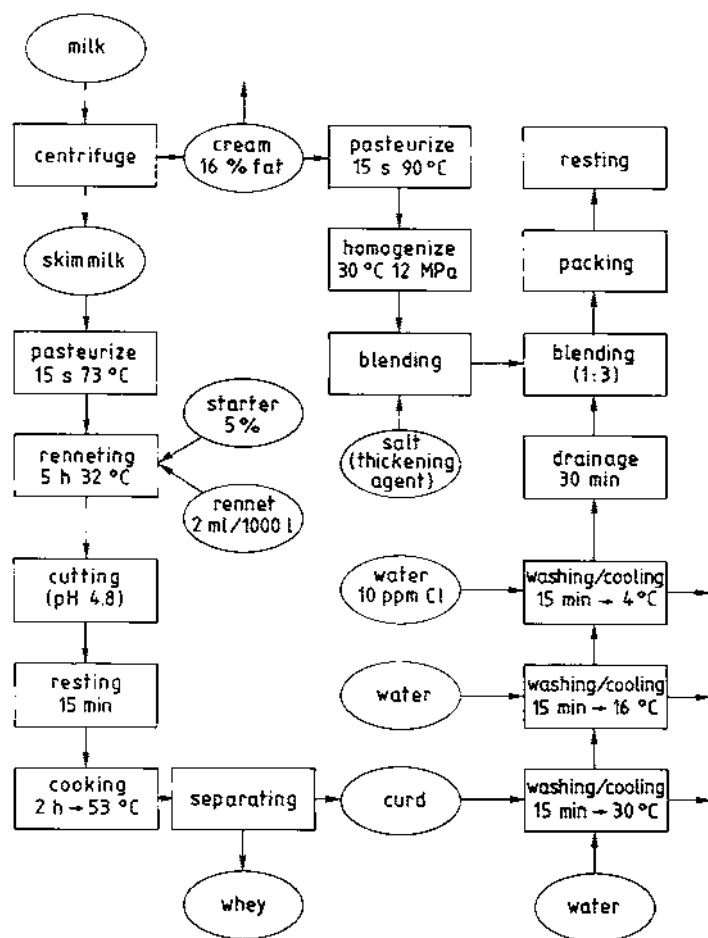
FIGURE 25.7 Example of the traditional short-set method for the manufacture of cottage cheese.

and suffer less damage during subsequent stirring. Consistency and firmness of cottage cheese greatly depend on the heating time during cooking. The longer it is, the more evenly all granules synerese, whereas fast heating up leads to curd granules with a dry and firm rind. To avoid fusing of the curd grains, the curd and whey mixture should be gently stirred at all times. The cooking temperature depends on the desired moisture content. During the first stage of cooking (say,

until 43°C) some additional acid is produced in curd and whey. If the curd is cooked to a relatively high temperature of 55–57°C and maintained for some time, many bacteria are killed and most of the rennet is inactivated.

Usually, the curd is washed 3 times, the quantities of water added being equal to those of the whey drawn off. If washing is less intense the acid flavor is insufficiently removed, although preferences vary. Often, some 5–20 ppm of activated chlorine is added to the last wash water to prevent growth of undesirable microorganisms.

Blending the low-fat curd with cream considerably improves the flavor of cottage cheese. The fat content of the cream may range from 10% to 20%. The cream is often homogenized, which increases its viscosity (Section 8.7). To arrive at a similar flavor perception a much smaller amount of cream is needed if it is added to the curd rather than to the cheese milk. Moreover, the loss of fat into the whey would be high with the latter method. The plasma of the cream is believed to be absorbed by the curd, so that specifically the fat globules remain on the outside of the curd granules to ''lubricate'' them. The ''absorption'' appears to take approximately 30–40 min. Properties of the curd granules, including size and water content, as well as the cream (fat content, viscosity, pH, treatments such as homogenization), determine the quality of the product. The product should not whey off or separate ''free cream.'' The low-fat curd can be blended with sweet or cultured cream. Cream with a high pH leads more readily to separation of liquid than cream with a lower pH. Well-soured cream (in which the casein is thus coagulated) does not give free cream. Sometimes a thickening agent is added to the cream to prevent the latter defect.

Attempts to preconcentrate the skim milk by ultrafiltration have met with little success. The curd acquires an undesirable consistency and fails to properly absorb the cream plasma. Continuous curd manufacture may be applied. The freshly cut curd is put onto a perforated conveyor belt, on which it first drains and is subsequently washed. Establishing the correct moisture content is possible by gentle pressing under rollers.

In spite of all precautions, cottage cheese does not have a long shelf life. Its composition permits growth of microorganisms. Very good hygiene during manufacture and packing are essential. Sorbic acid may be added to the creaming mix to improve the shelf life. Alternatively, the product may be packed under CO_2, due to which growth of gram-negative psychrotrophs is especially inhibited.

25.3   GOUDA-TYPE CHEESES

Gouda-type cheeses are ripened, fairly firm, sliceable cheeses made from fresh cows' milk. Mesophilic starters are used that generally produce CO_2, mainly from citric acid, thus causing formation of holes in the cheese. The cheese is pressed,

salted in brine, and has no essential surface flora. This type of cheese is made in large quantities. Gouda and Edam cheese are the archetypes, but there are numerous related or derived types in many countries. Accordingly, the properties vary widely as follows:

1. The water content in the (unripened) fat-free cheese ranges from 53% to 63%, and the consistency varies correspondingly from firm to rather soft.
2. The fat content in the dry matter mostly ranges from 40% to 52%, but there are related low-fat and high-fat types as well.
3. The loaf size may be between 0.2 and 20 kg; the shape may be a sphere, a flat cylinder, a rectangular block, or like that of a loaf of bread.
4. The maturation may take from several weeks to years; the consistency and above all the flavor vary correspondingly.
5. Sometimes substances are added in addition to salt, especially cumin.

25.3.1 Manufacture

Figure 25.8 is an example of a flow sheet for the production of Edam cheese in a fairly traditional way, whereas Figure 25.9 is an example of Gouda cheese making by a modern large-scale method. Some process steps will be discussed more fully.

Thermalizing serves to prevent psychrotrophs from forming lipase; strong hydrolysis of fat would cause a distinct off-flavor, mainly in young, i.e., little matured, cheese. Adverse effects of proteinases of psychrotrophs are not known.

Pasteurization is meant to kill pathogens and spoilage microorganisms (e.g., Fig. 22.1); spores of butyric acid bacteria and some streptococci are not killed. Most enzymes need not be inactivated, except lipoprotein lipase in some instances (young cheese); xanthine oxidase should remain intact because it is essential for the action of nitrate against butyric acid bacteria (see Section 24.2). In some countries it is illegal to pasteurize the cheese milk to the extent of significantly decreasing the protein content of the resulting whey. Usually the milk is pasteurized shortly before renneting, which reestablishes its rennetability (which has been considerably reduced during prolonged cold storage); moreover, it implies that the fat is again liquid, preventing churning of fat globules.

Bactofugation and addition of nitrate serve to prevent butyric acid fermentation. At present, the nitrate often is not added to the milk, but to the mixture of curd and whey after a good deal of the whey first has been removed. This is partly to obtain the largest possible amount of whey without added nitrate. A greater amount of nitrate should be added if bactofugation is omitted.

Starter (see also Section 11.4). Nowadays, starters are often made by transferring a deep-frozen concentrated culture to a closed vat with high-pasteurized skim milk, while contamination by phages is rigorously avoided. Subsequent
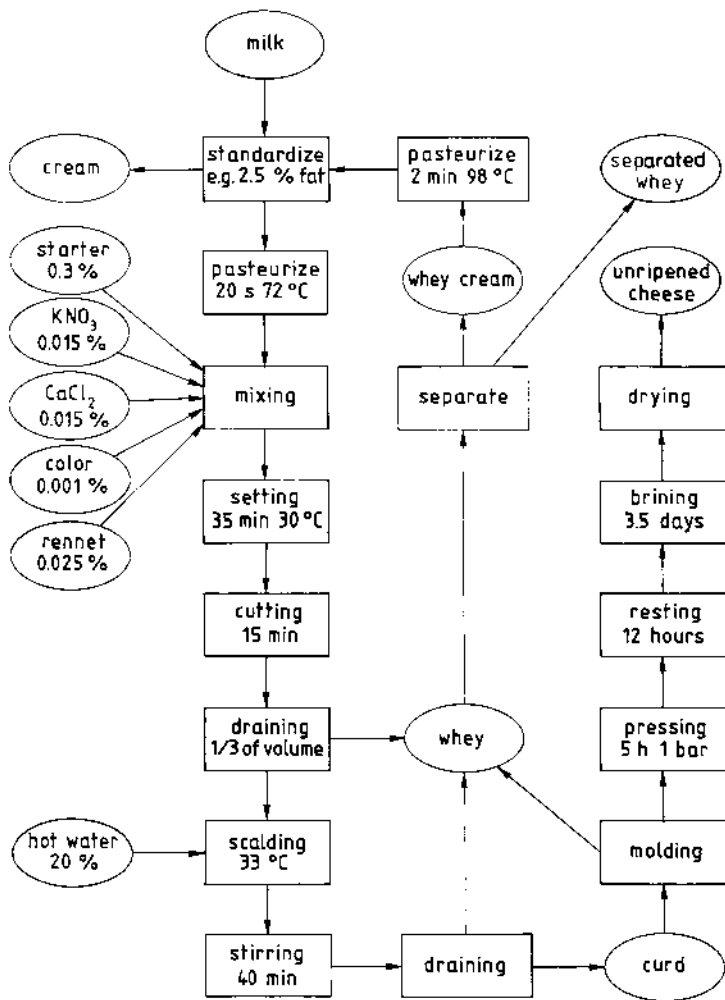
FIGURE 25.8 Example of a traditional method for the manufacture of Edam cheese.

culturing is for 16–18 h at 18–20°C. The quantity of starter added to the cheese milk is relatively large because a fast acid production in the curd is desirable. The added starter enriches the milk with, say, 10^7 bacteria per ml; the latter are concentrated approximately 10 times by the cheese making, while they multiply again by a factor of 10, i.e., about 3 generation times. This leads to the cheese at pressing containing about 10^9 starter bacteria per gram. Obviously, such a high
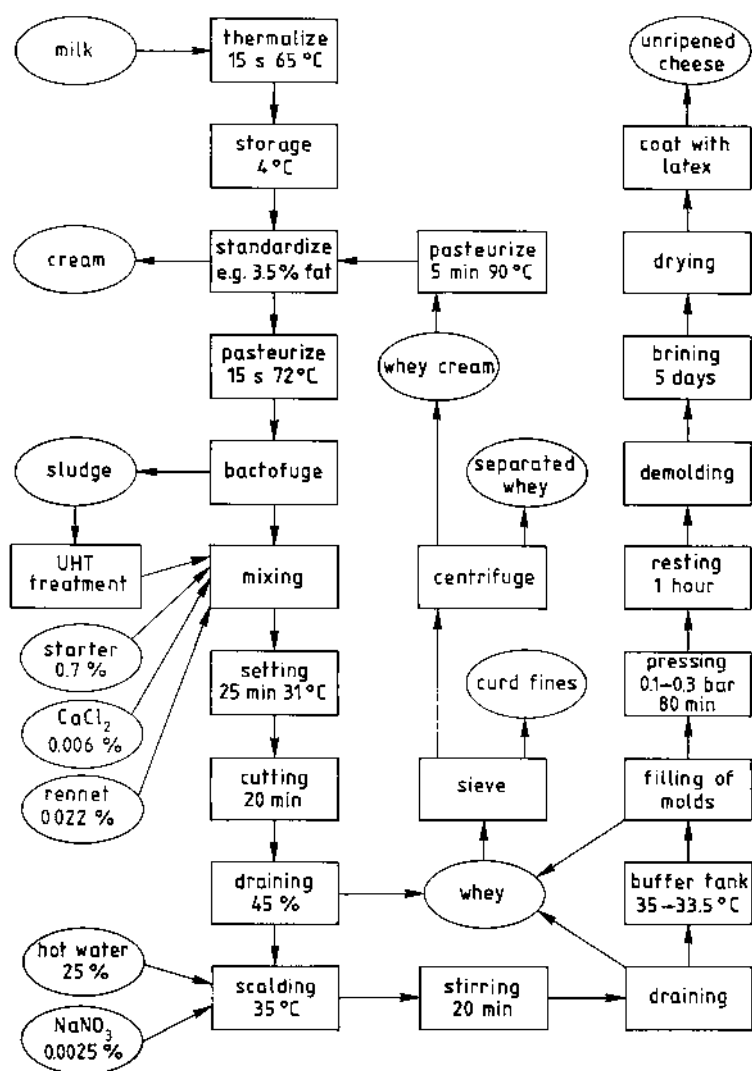
FIGURE 25.9 Example of the manufacture of 12-kg Gouda cheese by a modern method.

count is needed because at brining the cheese often contains an appreciable amount of residual lactose, while both the salt and the low temperature stop the growth of lactic acid bacteria, at least in the outermost part of the cheese. The residual lactose should then be hydrolyzed by the enzyme system of bacterial cells already present (see Fig. 25.10).
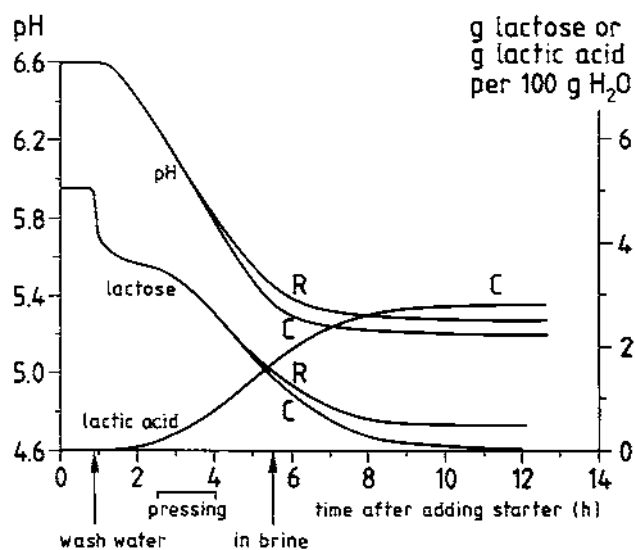
FIGURE 25.10 Acid production during Gouda cheese manufacture (conditions comparable to those in Fig. 25.9). Quantities of lactose and lactic acid, and the pH of curd and cheese, as a function of the time after starter addition. C, center of the loaf; R, rind portion. Data kindly provided by M. D. Northolt and G. van den Berg, NIZO.

Acidification of the curd should not only be fast, but reproducibly fast, to allow the making of cheese of constant composition. In the modern manufacturing process a fixed time schedule is maintained, making it virtually impossible to vary the process, e.g., to speed up or slow down syneresis. Because of this, the starter should be active and the cheese milk devoid of growth-inhibiting substances (antibiotics). No great harm will arise from a slight contamination of the cheese milk by bacteriophages if starters are used with a great number of different strains. In this situation it is highly improbable that more than a few of these strains will suffer from the phages and most of the bacteria can always grow satisfactorily. Moreover, released phages can hardly spread throughout the bulk of the milk if it has been clotted (see also Section 11.4).

The activity of the starter is checked by inoculating the cheese milk, along with a standarized high-heated (hence phage free) skim milk, with the starter and by culturing these milks; the respective rates of acid production should be sufficient. Even if this is all right, problems can still arise due to accumulation of phages. Obviously, any contamination of cheese milk and of whey + curd with remnants of a previous batch should be rigorously avoided. Whey cream, used

for adjusting the fat content of the cheese milk, should be intensely pasteurized; curd fines should not be put back into the vat; and the processing machinery should be thoroughly cleaned and disinfected after, say, any 10 h of use. The starter should be cultured under absolutely aseptic conditions.

Furthermore, the starters are selected on the basis of their capacity to produce appropriate amounts of CO_2 (presence of *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* and/or *Leuconostoc cremoris*) and on the basis of their proteolytic capacity. A proteolytic enzyme system causing bitter peptides, or too slow a breakdown of bitter peptides formed, obviously is highly undesirable.

The *washing* serves to adjust the sugar content of the cheese and hence its ultimate pH (see Section 22.2). It also affects the yield of the cheese. If washing is omitted (or if a fixed amount of water is used), every percentage unit higher water content of the cheese—attained, for instance, by stirring for a shorter time, or by using a lower scalding temperature—will lead to the pH of the cheese being approximately 0.1 unit lower.

Figure 25.11 gives the relation between the amount of curd wash water used and the lactose content of the cheese. This relationship obviously depends on the ultimate water content of the cheese and on the lactose content of the milk. Note that here ''lactose'' means lactose present plus lactose that already has (partly) been fermented to lactic acid. Furthermore, the desirable lactose content clearly depends on the pH of the cheese after complete conversion of the sugar. This pH is lowered by about 0.03 unit if the lactose percentage in the fat-free dry cheese is increased by 0.2 (corresponding to a 5% lower amount of wash water). Such relationships are approximative, since they also depend on other factors such as the rate of acid production and, of course, the efficiency of the above washing step. Usually, 90% of the lactose is supposed to be effectively washed out, but that must clearly depend on the duration of washing, the size of curd grains, and the intensity of stirring.

Control of the water content is most simply achieved by adjusting the duration of stirring or the scalding temperature. The stirring time is preferably kept brief for economic reasons; because of this a fairly high scalding temperature is used. The virulence of the starter bacteria significantly diminishes, however, if this temperature exceeds 35°C. Furthermore, the manufacturer will aim at achieving the narrowest possible range in the water content of the cheese, partly because it allows a closer approach to the statutory maximum. A complication arises when curd is made in large quantities because a considerable time elapses between the beginning of pressing the first and the last loaf of the batch, and during this period the water content of the curd continues to decrease. To counterbalance this decrease, the temperature of the curd + whey mixture may gradually be lowered (see Fig. 25.9). Alternatively, the molds filled latest can be the first ones put under the press (see Section 22.3).

Molding. Traditionally, the curd settled to a layer of uniform thickness,
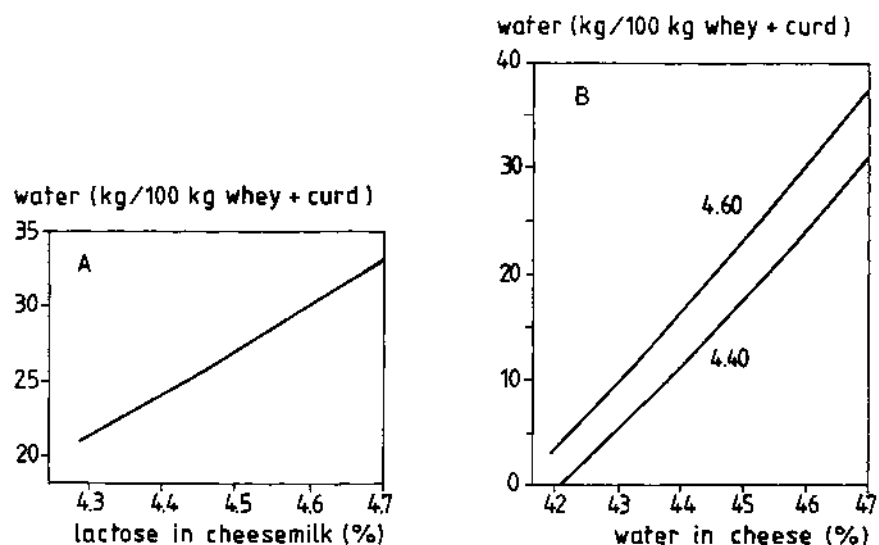
FIGURE 25.11 The amount of curd wash water needed to arrive at the pH desired for Gouda cheese (about 5.2). (A) Quantity of water to add, as a function of the lactose content of the cheese milk. (B) Same, as a function of the desired water content of the cheese before brining. Approximate averages for two different lactose contents of the milk (indicated near the curves); manufacturing conditions comparable to those in Figure 25.9. The curve in A refers to cheese with 46% water before brining. After G. van den Berg and E. de Vries, *Zuivelzicht* **68** (1976) 878, 924.

and it then was left for some time, resulting in a partial fusion of the curd grains (Section 22.3). The process can be accelerated by lightly pressing with perforated metal plates laid on the layer of curd grains in the whey. When the mass of curd has become sufficiently coherent, it is cut into blocks. The blocks are wrapped in cloth, put into (originally wooden) molds, and pressed for several hours at a pressure of 0.5–1 bar, which causes a somewhat thick and firm rind. After pressing, the cheese is left upside down in the mold for several hours for ''shaping,'' which causes the loaf to attain a more symmetrical shape. The resting mainly functions, however, to continue the acid production in the cheese, so that lactose is almost completely converted when brining starts, i.e., about 24 h after renneting. With time, this traditional method of manufacture (Fig. 25.8) has increasingly been shortened (except in the manufacture of farmhouse Gouda cheese).

In the modern large-scale manufacture (Fig. 25.9), the change is even more pronounced. The mixture of curd and whey is usually transferred to a vertical
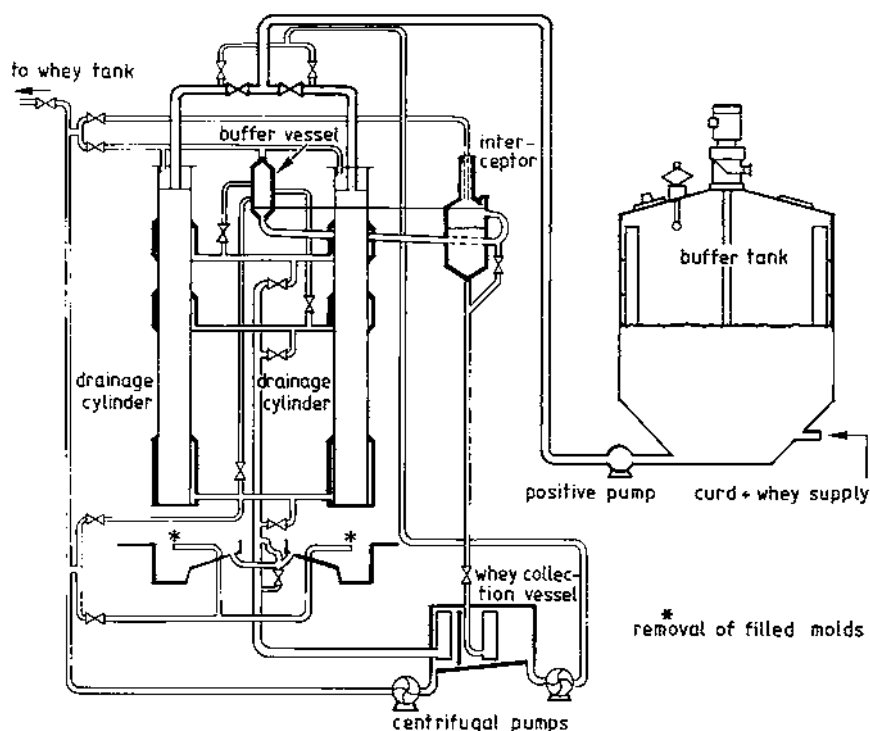
FIGURE 25.12 Diagram of machinery for the separation of whey and curd and for the formation of cylindrical loaves of coherent curd by means of drainage cylinders. Example according to the Casomatic system (Tetra Pak Tebel B. V.).

drainage and filling cylinder (Fig. 25.12). The curd is allowed to settle for a while, which causes a beginning fusion of the curd grains, especially in the bottom layer, where the pressure on the curd grains is highest. After some time, the lower layer of the formed column of curd falls in a mold and is cut off. The plastic mold is lined with gauze to ensure formation of a closed rind. The pressure is applied for a much shorter time and is lower than in earlier days. The cheese is put into brine within 6 h after renneting of the milk.

In both manufacturing processes described, the curd is under the whey while being made into a coherent mass. In this way, inclusion of air is largely prevented. If the cheese is to be very dry, the drained curd can be stirred again (be ''crumbled''), which causes considerable additional expulsion of whey (with a high fat content), but also inclusion of air pockets.

Brining is done to provide the cheese with salt (Section 22.4), to cool the

loaves, and to give them a certain firmness. The newly made cheese would readily deform under its own weight if it does not have a firm high-salt rind, especially if its moisture content is high.

An appreciable amount of lactose diffuses out of the cheese into the brine. This is because the cheese still contains lactose when salting starts; moreover, the conversion of lactose in the cheese rind is strongly impeded due to the high salt concentration. The lactose content of the brine mounts to about 0.5%.

Growth of microorganisms, mostly yeasts and lactobacilli (Section 24.3), may cause problems, especially if the temperature of the brine is too high (>14°C), the pH is too high (>4.6), or the concentration is too low (<16% NaCl). The pH is usually maintained below 4.6 by adding hydrochloric acid. Weak brine is sometimes used to allow the brining of Gouda cheese for exactly 1 week. This enables the use of a fixed daily time schedule in the plant. If the brine is too weak, especially if it contains too little Ca (freshly prepared brine), the rind of the cheese softens and swells; in more serious cases, it becomes slippery or slimy and the cheese starts to dissolve. Obviously, this must be avoided.

The cheese absorbs a certain amount of salt during brining, while at the same time losing a far greater quantity of moisture. This causes a net weight loss of cheese. The amount of expelled moisture is, say, 4 times that of salt taken up. The volume of the brine thus increases, leading to an excess of brine, which has to be discharged, causing environmental pollution. Discharging this excess, on the one hand, and adding salt to the remaining brine, on the other, essentially is an illogical operation because only the water released from the cheese into the brine is superfluous. Removal of water from the surplus brine can be achieved by evaporation or electrodialysis, and by readdition of the concentrate to the brine. This method obviously results in decreased pollution.

The rind treatment (see Section 22.5) often implies providing the cheese with a layer of a latex emulsion. It primarily prevents mold growth on the rind, but it also protects against mechanical damage, which implies that even a young cheese with a weak rind can be handled. Successive treatments with latex should be applied, and it should contain fungicides, e.g., natamycin.

Gouda cheese is sometimes made in rectangular loaves for curing while wrapped in shrink film (e.g., Saran). The water content of the cheese immediately after its manufacture should be slightly lower (because less water evaporates during maturation). The cheese is stored at a somewhat lower temperature (e.g., 10°C); otherwise an ill-balanced flavor develops, the cause of which is not clear. Furthermore, a different starter is generally used.

25.3.2 Properties and Defects

Gouda-type cheese can vary widely with respect to loaf size, shape, water content, and maturation time. A smaller cheese is usually adjusted to a higher water con-

tent and matures for a shorter time. At present, most Gouda-type cheese has a semihard consistency, which means that it is fairly firm but readily sliceable. It is not too mature (usually from 2 to 5 months), and hence the flavor is not very pronounced. Greater variation is especially found in farm-made cheese. Very mature (aged) cheese can have a very piquant flavor and has a short, often somewhat crumbly consistency. If the cheese is to be matured for a long time it should be made differently (larger, somewhat drier) from a cheese that is to be consumed while young.

The pH of the young cheese often is between 5.0 and 5.5; that of some types of Edam is 4.9, whereas the pH of some farm-made cheeses is rather high. Cheese manufacture in the plant usually aims at a pH after brining of 5.2. A higher pH implies an increased risk of defects due to growth of undesirable bacteria. The pH markedly affects maturation. At a low pH hydrolysis of fat often is predominant, whereas the formation of flavor compounds due to protein degradation usually is stronger at a higher pH. Proteolysis is mainly due to the action of rennet and enzymes of starter lactococci. Different starters cause differences in flavor. They may also cause differences in consistency (see further Sections 23.3 and 23.5).

25.3.2.1 Eye Formation

The reader is also referred to Section 23.6. The cross-section of most of the cheeses, except small loaves, should exhibit a few spherical and shiny holes of some 5–10 mm in diameter (eyes). ''Blind'' cheese, cheese with many holes or with large holes that are irregular in shape, and cheese with slits or cracks are considered defective. The formation of holes is mainly due to the production of CO_2 from citric acid by citric acid fermenting starter bacteria. Most CO_2 is formed within a week after manufacture, whereas the eyes develop from, say, 1 to 4 weeks, depending on the type of starter applied. L starters, in which leuconostocs are the only CO_2 producers, form less CO_2 than do DL starters (which contain *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*) and at a slower rate. Accordingly, L starters more readily cause a blind cheese.

The presence of cracks or slits in the cheese mass represents a problem, partly because cutting of the cheese into coherent slices then is hardly possible. Cracks form if the gas pressure in a hole exceeds the fracture stress of the cheese mass. This is enhanced by:

  a. *A high gas pressure*. This can arise especially when gas is formed at a high rate (by a strongly gas-forming starter, by gas-forming contaminating bacteria, or due to a high temperature).
  b. *A low fracture stress*. The fracture stress will usually be low if the cheese consistency is short, which means that the cheese fractures at a small deformation (Section 23.6). This can be expected when the cheese pH is low (say, <5.1), its salt content is high (because of this,

cracks often form in the outermost part of the cheese), and the protein has been extensively degraded. If the pH is high (>5.4) cracks can also form, since the cheese consistency becomes, again, shorter at a pH higher than about 5.3; moreover, proteolysis (caused by other than rennet enzymes) may be faster.

Excessive gas production (leading to cheese with many or very large holes) is caused by abundant growth of contaminating bacteria (Chapter 24). As a rule, this is a serious defect, partly because it is usually associated with a marked off-flavor. Butyric acid fermentation often causes the cheese to become unsalable.

25.3.2.2   Defects

Some defects have already been mentioned above (Section 23.8, Chapter 24). Most defects are of microbial origin, but cheese composition (hence, errors in cheese manufacture) can considerably affect the growth potential of undesirable bacteria, e.g., salt, water, and sugar content, and pH. More bacteria can grow if the pH is higher, especially if unfermented sugar is left. Because of this, an inadequate acid production, which is usually caused by contamination by bacteriophages, favors defects. Strict hygiene is paramount. Figure 25.13 locates possible sources of contamination.

In cheese made of pasteurized milk the main defects include:

- Butyric acid fermentation (Section 24.2).
- Off-flavors, usually caused by excessive growth of lactobacilli (Section 24.3)
- A bitter flavor, which can develop when the cheese retains much rennet while the starter bacteria form too many bitter peptides and decompose them insufficiently (see Section 23.5).
- Cracks in the cheese mass (see above).
- Mold growth on or in the rind (Section 24.6).
- Shape and rind defects (e.g., due to rough handling)

In cheese made from raw milk several bacteria (including coliforms, propionic acid bacteria, fecal streptococci) can grow and thereby cause defects. In addition to gas production (early and late blowing), off-flavors such as sour, yeasty, putrid, fruity, and H_2S flavor can appear. The cheese can also turn rancid (soapy) due to milk lipase activity, especially if the milk fat globule membranes have been damaged during milk handling. Young cheese made of pasteurized milk can show a rancid flavor if the milk was already rancid before pasteurization (often due to bacterial lipases), but usually little lipolysis occurs.

Obviously, Gouda cheese will rarely have off-flavors if it is very hygienically made from good-quality pasteurized milk; but this also implies that its flavor may be rather flat (unless the cheese is matured long enough) and, above all,
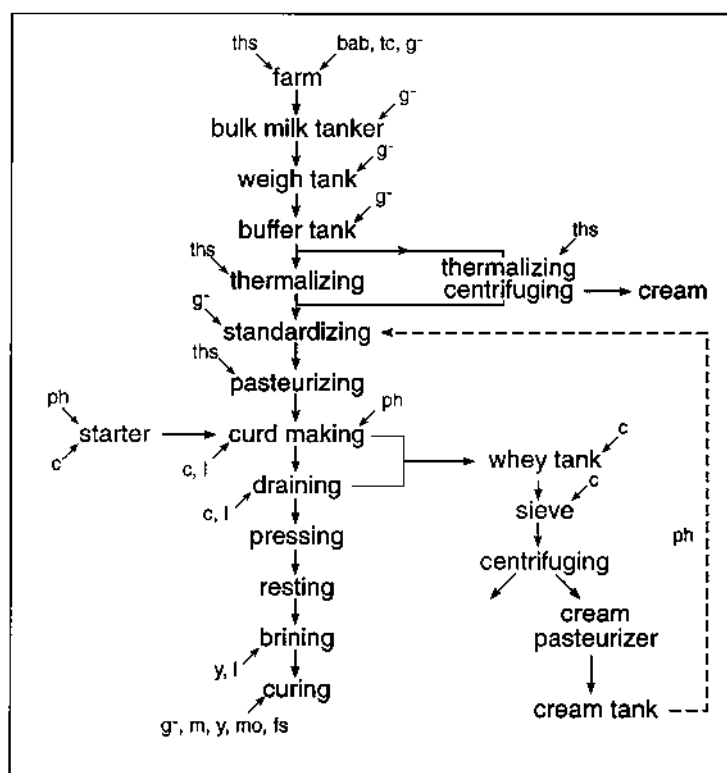
FIGURE 25.13 Points where contamination by microbes and disturbing phages can occur, in the case of Gouda cheese manufacture starting with modern milk collection (it thus involves cold storage of farmer-delivered milk) up to maturation. Abbreviations: bab, butyric acid bacteria; c, coliforms; fs, fecal streptococci; g−, gram-negative rods (including psychrotrophs); l, lactobacilli; m, micrococci; mo, molds; ph, bacteriophages; tc, total count; ths, thermophilic streptococci; y, yeasts. After M. D. Northolt, *Voedingsmiddelentechnologie* **20** (1987) 17.

hardly varying. Only differences between starters then can cause differences in flavor.

25.4 SWISS AND PASTA FILATA TYPES

In the manufacture of these types of cheese high temperatures (50°C or higher) are applied during the curd treatment (see Fig. 25.1). The use of such high temperatures has obvious effects on the properties of the cheese:

a. The cheese becomes somewhat dry and not very acidic, despite the absence of a washing step during the curd making.
b. Many potentially harmful bacteria are killed at high temperature, which is maintained for a considerable time, often more than 1 h. For most of the bacteria, the decimal reduction time is 2–50 min at 50°C. This implies that thermalization or even pasteurization is essentially applied and pasteurization of the cheese milk can thus be omitted.
c. A considerable proportion of the rennet is inactivated during curd treatment, which affects proteolysis in the cheese.
d. Mesophilic lactic acid bacteria are for the greater part inactivated. Accordingly, the lactic acid (starter) bacteria involved are predominantly thermophilic.

Nowadays starters are used that are grown at high temperature, e.g., 40°C, and that are mainly composed of strains of the thermophilic *Streptococcus thermophilus*, *Lactobacillus helveticus*, *L. lactis*, and sometimes also *L. delbrückii* ssp. *bulgaricus*. The mesophilic *Lactococcus lactis*, ssp. *lactis* and *cremoris*, may occur as a minority flora. The starter bacteria are homofermentative; they produce little if any CO_2 at the initial stages of fermentation; they cannot hydrolyze galactose or do so poorly (Section 11.1); and they develop a type of proteolysis (and thus a flavor in the mature cheese) that differs from that of mesophilic starters. The lactobacilli are fairly proteolytic; *S. thermophilus* is less so.

Originally, these types of cheese were made predominantly in the Alps and in Italy, but some types now are spread far wider. There are several variants. A kind of archetype is Bergkäse (mountain cheese), also designated Alpkäse or Beaufort. In summer, the dairy cattle were transferred from the village to the elevated mountain meadows (Alm), where the milk was made into cheese. Therefore, the manufacture of the cheese should be simple; the cheese should be easy to handle and very durable. These are somewhat dry, still sliceable, quite large (20–40 kg), flat-cylindrical cheeses that mature for several months or longer. On the rind of some of these cheeses a layer of salt-tolerant yeasts and bacteria is grown (red smear, Rotschmiere, ferments du rouge; see Section 25.6.1), e.g., Gruyère (Switzerland) and Comté (France). The latter cheeses often are somewhat larger. The variant evolved in German-speaking Switzerland is Emmentaler (often simply called ''Swiss''), which is still larger (up to 130 kg), contains little salt, and does not have a read smear; it has a distinct propionic acid fermentation. Still drier types, usually quite large high-cylindrical cheeses, are Sbrinz (Switzerland) and in Italy Parmesan (Parmigiano Reggiano and Grana Padana); a related type is Pecorino Romano, made of ewes' milk. These cheeses should mature for years, becoming very hard over that time. They are grating cheeses, to be used in cooking. Parmesan cheese has a reduced fat content (say, 35% fat in the dry

matter), with the reduction achieved by gravity creaming of the milk. With the cream layer many bacteria and spores, such as butyric acid bacteria, are also removed.

Traditionally, in the south of Italy, as well as in other regions around the eastern part of the Mediterranean, pasta filata, i.e., stretched curd, cheeses are made. The curd is intensely heated and kneaded into loaves of small to medium size, and of several shapes. An almost unripened cheese is mozzarella (''pizza cheese''). Matured types are provolone and caciocavallo, in the Balkans known as kashkaval. Some of these cheeses are smoked, which may prevent mold growth.

Two types of cheese that are nowadays made in large quantities will be considered in more detail.

25.4.1 Emmentaler

This is a very large cheese, weighing 60–130 kg; the loaf shape is flat cylindrical, at least initially. The extensive gas production causes the cheese to bulge upward, whereas its great weight flattens it slightly leading to bulging sides. The cheese has many round eyes, 1–4 cm in diameter, and a smooth, long, somewhat rubbery texture. The flavor is mild and rather sweetish. The composition after 3–4 months of maturation is approximately as follows:

Protein	29%, including protein degradation products
Fat	31% (48% in the dry matter)
Water	35% (51% in the fat-free cheese)
NaCl	0.5% (1.4% in the water)
Propionic acid	0.7%
CO_2	0.2%

Figure 25.14 outlines the manufacturing process. The pH at 2 days usually is 5.1–5.3, slightly increasing again afterward. For the course of the temperature, see Figure 25.1. The maximum temperature is often 52–54°C. The higher it is, the less trouble there is with defects caused by raw milk of poor microbial quality. The milk is hardly ever pasteurized. Traditionally, the curd was made in copper vats, causing considerable contamination of the milk by copper; hence, the cheese contained up to 15 mg Cu per kg. The copper was believed to be necessary for a satisfactory flavor development, but this has never been shown conclusively. Presently, the curd is usually made in stainless steel vats (sometimes a copper salt is added). The copper contamination caused problems because the butter, made of the whey, rapidly developed autoxidation defects.

For Emmentaler cheese the growth of propionic acid bacteria is paramount. As a rule, a little of a culture of *Propionibacterium freudenreichii* ssp. *shermanii*
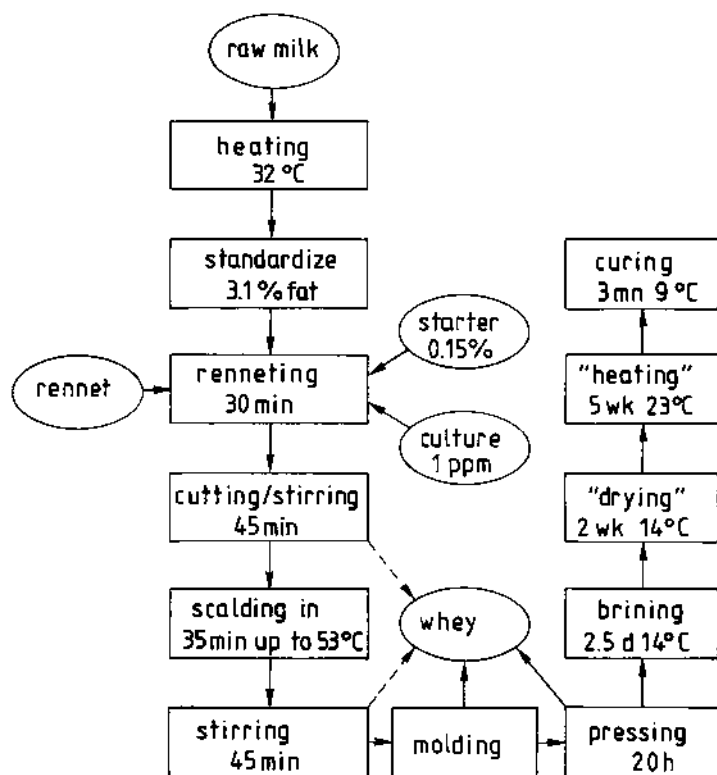
FIGURE 25.14 Example of the manufacture of Emmentaler cheese. The culture is of propionic acid bacteria. One thousand liters of milk yields one loaf of 81 kg. The ensuing whey yields about 10 kg whey butter.

is added to the cheese milk. These bacteria grow in 20–30 days to 10^8–10^9 cells per gram of cheese and ferment lactic acid approximately according to:

$$3 \, CH_3 \cdot CHOH \cdot COOH \rightarrow 2 \, CH_3 \cdot CH_2 \cdot COOH + CH_3 \cdot COOH + CO_2 + H_2O$$
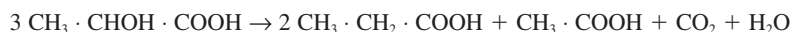
As is shown in Figure 25.15, almost all of the lactic acid is consumed. This causes the flavor of the cheese to change (propionate gives a sweetish taste) and large eyes to form. To secure a satisfactory fermentation to propionic acid, the curing temperature should be raised to 22–25°C for about 5 weeks. The production of CO_2 is controlled by varying the temperature and the length of time in the ripening room. The amount of CO_2 produced in an 80-kg cheese approximates 120 L, 60 L of which dissolves, 40 L of which diffuses out of the loaf, and 20 L of which finds its way into the eyes; this implies that the volume of the cheese

mmol/kg
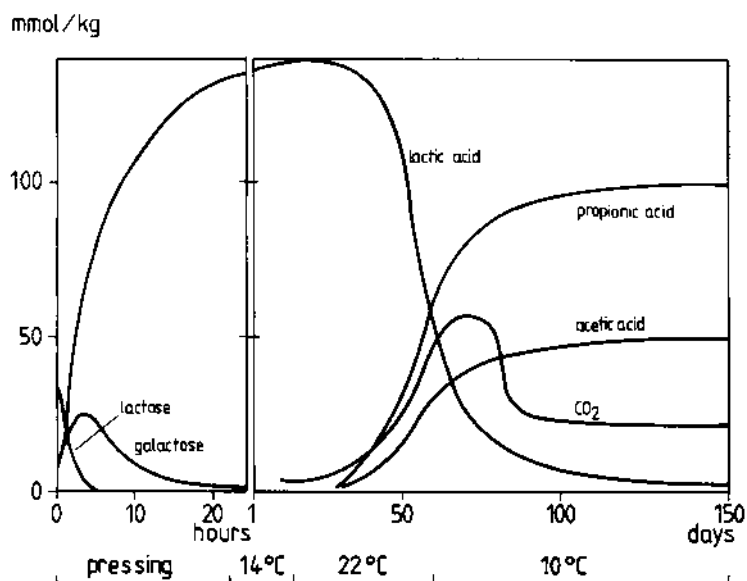
FIGURE 25.15 Fermentation of sugar and of lactic acid in Emmentaler cheese during and after pressing. All quantities are in mmol per kg cheese; quantities in the holes are not included. After Steffen et al., In P. F. Fox, ed., *Cheese*: *Chemistry, Physics and Microbiology*, Vol. 2, *Major Cheese Groups*, 2nd ed., 1993.

increases by almost 25%. Before entering the curing room, the cheese is kept cool for some time, which allows the salt to partly diffuse throughout the cheese; furthermore, the keeping causes the cheese to attain a firm rind. During this time proteolysis should be limited because cracks would form rather than eyes during the ensuing CO_2 production.

Enzymes of the lactobacilli and plasmin from the milk are mainly responsible for proteolysis. Proteolysis is less than and different from that in Dutch and Cheddar-type cheeses. The consistency of the cheese remains fairly long, i.e., the true deformation (natural strain) at fracture in a mature cheese approximates 1.2 (cf. Fig. 23.9). Usually, the level of proteolysis is higher in the rind portion than in the center. This is ascribed to the long time needed for the center of the cheese to cool after pressing. Consequently, in the rind a greater proportion of rennet may remain active and mesophilic lactic acid bacteria may grow better. Accordingly, cracks, if present, are usually observed in the rind portion of the cheese. The latter phenomenon may also be affected by the higher salt content in the rind.

Possible defects:

Growth of *Clostridium sporogenes* causes an offensive flavor. Hygiene dur-
    ing milking and milk collection is the remedy.

Lipolysis should be slight.

C. tyrobutyricum and, to a lesser extent, *C. butyricum* can grow very well
    in the cheese and spoil it completely. Control by means of addition of
    nitrate does not apply because the quantity of nitrate needed to suppress
    butyric acid fermentation would also suppress propionic acid fermenta-
    tion. (Note that the cheese pH is fairly high and the salt content low.)
    Because of this, dairy farmers delivering milk for the manufacture of
    Emmentaler cheese are not allowed to have silage.

Cheese with numerous tiny holes can result from growth of heterofermenta-
    tive lactic acid bacteria.

Enterobacteria can also cause cracks and an off-flavor. They are killed by
    adequate scalding.

Some lactic acid bacteria, especially mesophilic lactobacilli, can eventually
    produce a considerable amount of CO_2 by decarboxylation of amino
    acids. Cracks can form because in the meantime the cheese texture has
    become shorter. Moreover, biogenic amines can form.

To an extent, Jarlsberg cheese is related to Emmentaler. It is of Norwegian
origin. In other countries it may have other designations, e.g., Maasdammer
(Dutch). The cheese resembles Gouda in many respects, i.e., size, water content,
pasteurization of the cheese milk, use of mesophilic and partly heterofermentative
starters, and a scalding temperature of about 35°C. However, propionic acid fer-
mentation does occur (a culture is added). For this to happen, the pH should be
relatively high, which is achieved by washing the curd. For a satisfactory flavor,
the salt content should be fairly low. All in all, conditions are highly favorable
for butyric acid fermentation. Three measures are taken to control it:

- Bactofugation of the cheese milk
- Addition of $NaNO_3$
- Addition of a culture of a special strain of *Lactobacillus delbrueckii*
  ssp. *bulgaricus*, which can reduce NO_3 to NO_2. NO_2 is even further
  reduced because too much NO_2 would inhibit the propionic acid fermen-
  tation.

25.4.2 Mozzarella

Mozzarella is a small cheese, weighing 50–400 g. The loaf shape is a somewhat
flattened sphere. The cheese is mostly used in cooking, especially on pizzas. The
flavor should not be pronounced and the cheese should have adequate melting

properties, i.e., soften on heating, become smooth, and flow. Originally, it was mainly made from buffalo milk. Nowadays it is usually made from cows' milk, and in Italy from mixtures. The cheese contains 35% to 45% fat in the dry matter, 52% to 56% water, and about 1% salt. Being a few days old, it is little matured and has a rather soft and long consistency.

An example of a manufacturing scheme is given in Figure 25.16. How-
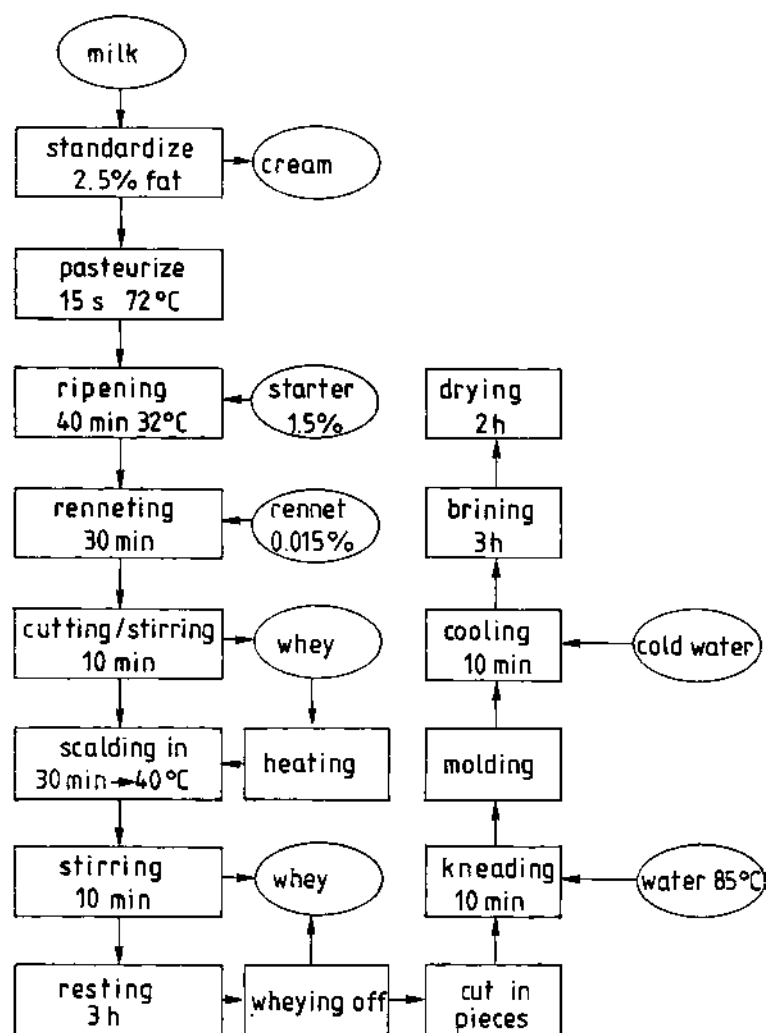
FIGURE 25.16 Example of the manufacture of mozzarella. 100-g loaves.

ever, there are several different manufacturing procedures for mozzarella and related types. Coagulation by adding acid even occurs. The starter is mainly composed of *S. thermophilus, L. delbrückii* ssp. *bulgaricus*, and *L. helveticus*, although some mesophilic lactococci may be present. The lactobacilli produce acid in the cheese, even after the stretching process. A decrease of the pH of the curd to 5.2–5.3 when kneading starts is paramount because otherwise ''stretching'' is not possible. If the pH is above 5.4 the cheese mass is too firm; if the pH is below 5.1 it is too crumbly. To arrive at the desired pH in the curd, various manufacturing processes are applied, i.e., simple resting or a kind of cheddaring process. The long manufacturing time, the high temperature (needed to kill unsiderable bacteria), and the significant acid production would readily cause the moisture content of the cheese to become too low. Because of this, the initial curd treatment often is very brief. After that, the curd settles and fuses, due to which the outflow of whey is considerably slowed down. The temperature is increased only after fusion is complete.

Then the curd is kneaded. Traditionally, the proper moment was fixed by testing if a string could be drawn from a heated curd piece, i.e., pasta filata. The ''ripened'' curd mass can be cut into strips, or it can be coarsely milled as in Cheddar cheese manufacture. The slabs or strips are put in hot water and kneaded, which may be done by hand. The kneading implies stretching and shearing of the curd mass; its main function is to rapidly increase the temperature of the whole mass to the desired level of about 55°C. The kneading also appears to slightly slow down syneresis (which may rapidly proceed at high temperature) and to affect the texture of the cheese; the cheese mass appears somewhat layered. Finally, lumps of curd are kneaded to the desired shape and placed in cold water. Usually, the cheese is not pressed.

During storage some proteolysis can occur, i.e., degradation of α_{s1}- and of β-casein. The relative contributions of chymosin and plasmin are uncertain because the extent to which rennet is inactivated is not quite clear. A low level of proteolysis enhances good melting characteristics. Often, the melting quality is a problem, the exact cause of its variation being unknown. Furthermore, the cheese mass may appear too transparent after melting, especially if its fat content is low. A further defect is brown discoloration. It is caused by Maillard reactions that occur during kneading, and later during melting, if galactose has not been sufficiently fermented by the starter bacteria.

Provolone is closely related to mozzarella as to the way of manufacture. It is made slightly drier and into larger loaves that weigh, for example, 5 kg, and they are of a different shape (e.g., an oblong pear). The salt content is somewhat higher and the cheese is cured for several months during which time significant proteolysis and lipolysis occur. Often, a lipase preparation is added to the cheese milk, which may cause a rather sharp flavor.

25.5 CHEDDAR-TYPE CHEESES

Cheddar-type cheeses are characterized by the mixing of salt with the curd before pressing it into a coherent loaf. Salt considerably retards the growth of lactic acid bacteria. Because of this, most of the lactose in the curd should have been converted before the curd is salted (and thus curd making requires a long time). Moreover, salted curd tends to fuse poorly during pressing if its pH is still too high (above, say, 5.6) because the curd flows insufficiently (Section 22.3).

Formerly, when cheese was made from skimmed milk, the milk was mostly left for creaming for such a long time that it turned sour. Naturally, the curd was also acidic and could thus be salted before pressing; an example is Frisian cheese. However, currently most cheeses of this type are made of unsoured milk, such as Cantal and almost all British types. The cheese becomes relatively dry due to the long curd-making time and to the low pH. Since the salt is relatively homogeneously dispersed through the fresh cheese, it can be made in large loaves, which is desirable to prevent water loss by evaporation and to minimize curing costs. On the other hand, it takes a long time for the interior of the loaf to cool.

Obviously, this involves typically hard cheeses with a long shelf life and without a surface flora. The best known is Cheddar: about 50% fat in the dry matter, not more than 38% water, originally of cylindrical shape, weighing about 30 kg; nowadays, rectangular blocks of variable (often large) size are predominantly made. Cheddar and derived varieties are now made all over the world, though primarily in English-speaking countries. Cheshire cheese is slightly more acidic and has a somewhat higher water content. The same holds for Caerphilly, but this cheese is eaten while young and is mainly used in cooking. Stilton cheese is quite different: The salted curd is not heavily pressed and shaped into a cheese with an open texture, and the cheese becomes veined with blue mold (Section 25.6.2).

Manufacture and properties of Cheddar will now be discussed in more detail.

25.5.1 Manufacture

Figure 25.17 outlines the manufacturing process (see also Figs. 25.1 and 25.3). It represents a somewhat traditional way of manufacture, though formerly the time from renneting to milling often was even much longer. The endpoint of the curd treatment in the vat (including ''cheddaring'') was assessed by determining the acidity of the whey. Nowadays a fixed time schedule is usually maintained and the processing time is much shorter, e.g., 3 h from renneting to milling. Figure 25.18 gives examples of the time scales involved in traditional and modern processes. Manufacture has also been mechanized to a high degree. Presently,
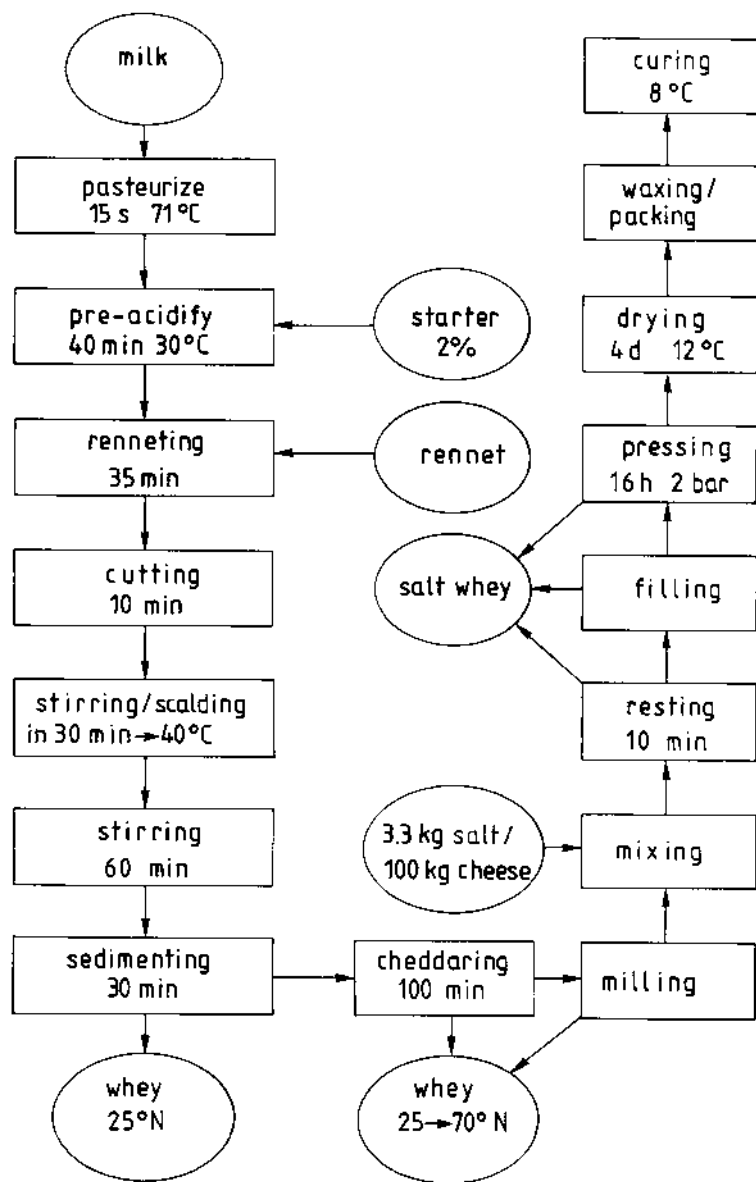
FIGURE 25.17   Example of a traditional method for manufacture of Cheddar.
Simplified.
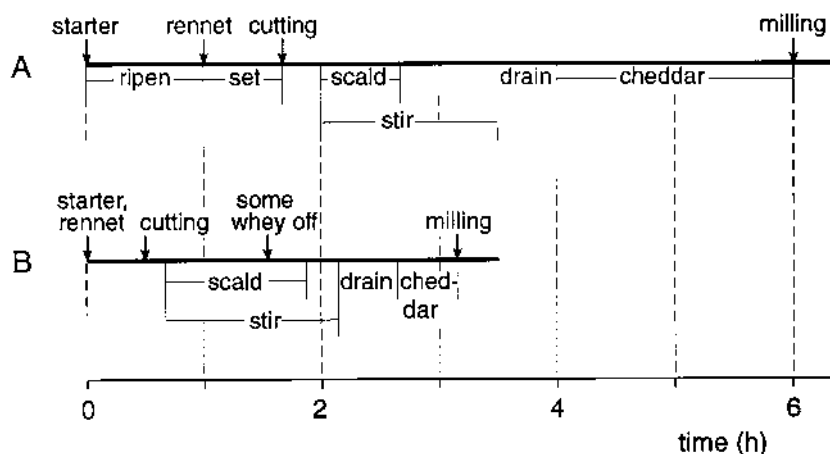
FIGURE 25.18 Time scales involved in the manufacture of Cheddar, up to milling. (A) A fairly traditional process. (B) A modern and fast manufacturing scheme.

the cheese milk is generally pasteurized, though a slightly milder heat treatment can be applied. Preacidification is now little practiced; however, a high percentage of starter is added.

Great demands are placed on the *starter*. The curd making should be as brief as possible for economical reasons, and this requires very fast acid production. Therefore, fast-growing, fast acid–producing, and phage-resistant bacteria are needed. This combination of requirements is hard to fulfill; for that reason, mixtures of single-strain, fast starters are generally employed, and contamination of the starter by phages is rigorously prevented (see Sections 11.3 and 11.4). Furthermore, the starter should be homofermentative (little gas production), and cause no bitterness in the cheese. Strains of *Lactococcus lactis* var. *cremoris* are usually used. Currently, a mixture of two strains is often applied, e.g., in a ratio of 1:2. The former strain is fairly heat-tolerant (i.e., it keeps growing during scalding) and is responsible for a fast acid production; however, these bacteria form many bitter peptides and decompose these poorly. The second strain is far less heat-tolerant (it does produce some acid during scalding, but it does not keep growing, and hence contributes little to the rate and extent of acid production), but has considerable ''debittering'' properties while not producing many bitter peptides itself; hence, the proteolytic system of these bacteria is of great importance for a satisfactory maturation.

After cutting, stirring, and scalding, the curd settles and fuses into a rather compact mass. Then *cheddaring* starts, which is (or was) a process, characteristic

of Cheddar and of most of its related types. The whey is drained off and the curd mass cut into large strips that are piled up. The slabs fuse again and are allowed to spread slowly into thinner slabs that are turned, cut again into strips, piled up, etc. This used to require much labor because it had to be done in such a way as to prevent the curd from cooling unevenly and too fast, thereby ensuring a uniform and fast acid production. The current mechanized cheddaring processes require less time and far less labor than the traditional method.

The curd mass will only flow readily if its pH is lower than 5.8 and the temperature is not too low. The flow causes a ''fibrous'' curd structure. It has long been assumed to be essential for obtaining a characteristic Cheddar and, accordingly, former manufacturing processes with mechanized curd manufacture included a spreading step. Later on, traditional cheddaring turned out to be unnecessary because the cheese does not show whether the step has been applied or not. To be sure, the deformation and piling of the curd squeezes out entrapped air, which favors the cheese to obtain a close texture. However, this can also be achieved by pressing the curd under vacuum, which currently is common practice. Furthermore, the flow of curd slightly hinders syneresis. Obviously, this effect should be considered in establishing the moisture content of the cheese.

Paramount is the acid production during cheddaring. When salting starts, the lactose content should not be over 0.6%, while the pH should preferably be 5.3–5.4. Water content and pH of the curd at that stage largely determine the composition of the cheese, i.e., water content, pH, amount of residual rennet and of calcium phosphate. (Note that Cantal cheese is often made in a different way. The curd is pressed into a loaf when its acidity is low. The ''cheese'' is subsequently ripened for a few days at 12–15°C. The unsalted cheese then is cut into pieces and treated as milled and cheddared curd.)

Prior to *salting*, the curd is milled, i.e., cut into strips about the size of a finger. Milling too finely leads to excessive loss of fat and of curd fines in the press whey. Milling too coarsely causes the diffusion of the salt into the strips to take a long time, resulting in a nonhomogeneous cheese texture (presumably because lactic acid bacteria are killed locally). The salt is mixed with the curd, and 10 min is allowed for salt absorption (''mellowing''); otherwise, excess salt would be lost with the whey, which in normal cases already contains about 50% of the added salt (both milling and pressing cause a considerable expulsion of whey). The salt should be evenly distributed, but this is hard to achieve. Acid production in the curd is insufficient if the cheese contains over 5% to 5.5% salt in the water, while at less than 4.5% salt the lactic acid bacteria ferment too fast. In either case the flavor development is unsatisfactory and contaminating organisms have a greater chance of growing out, which may cause strong off-flavors.

Formerly, *pressing* took a long time and pressure was high (about 2 bar), which was needed to achieve a close texture. Currently, the curd is usually

pressed under vacuum and lower pressures are applied. The lower the temperature and the pH during pressing, the more difficult it is to transform the salted curd into a coherent loaf. In Cheshire cheese making, the pH of the curd at salting and pressing is relatively low (about 5.1); accordingly, this cheese typically has a crumbly (very short) texture.

Soon after pressing, the cheese (often in the shape of large blocks) is supplied with a surface coating (e.g., paraffin), after which it needs little further care; the cheese loaves are often immediately packed in cases or boxes. Usually, the cheese is cured at low temperature (Table 22.3).

A modern variant of Cheddar cheese is *stirred curd* or *granular* cheese. The curd in the whey is continuously stirred until sufficient acid has been produced. Whey is separated from the curd under vacuum, the curd is salted, and the salt–curd mixture is pressed in molds. This procedure leads to a ''normal'' Cheddar with a close texture. Types of cheese have also been developed having a somewhat higher water content and pH, e.g., Colby (40% water) and Monterey (42%). Obviously, the curd should be washed; otherwise the pH becomes too low. Cold water should be used; otherwise the cheese becomes too dry. These cheeses are usually not pressed under vacuum and, accordingly, are open-textured; the mechanical holes increase slightly in size as a result of CO_2 production. In fact, such cheese is much like a Gouda. It has been claimed that even ''normal'' Gouda cheese can be made in a way similar to that of Cheddar cheese, i.e., by means of salting the curd.

25.5.2 Properties

Traditionally, Cheddar was a fairly acidic cheese—pH about 4.9—but presently a pH of 5.2 and even 5.3 is common (especially outside England). Its consistency is rather firm and short, at least if the pH is not too high and marked proteolysis has occurred. At the higher pH values, the consistency is more like that of Gouda cheese. The salt content also has a considerable effect: The cheese is too hard if the content is over 6% in the water, whereas at less than 4% it is too soft (maybe almost spreadable).

Cheddar cheese contains little active milk proteinase, active rennet (though less than most Dutch-type cheese), and a large pool of proteolytic enzymes from lactic acid bacteria; most of the fast acid–producing strains are also strongly proteolytic. At the low curing temperature (usually below 10°C) the proteolysis in the depth is relatively slow, whereas the degradation in the width is fast (in Section 23.3 the effects of cheese composition and of temperature on proteolysis by rennet enzymes are discussed). For instance, we have in cheese at several weeks old:

At pH 5.1: no α_{s1}-casein left, 55% β-casein left
At pH 5.3: 30% α_{s1}-casein left, 80% β-casein left

Cheddar is a cheese that may be cured for varying lengths of time, say, from 2 to 10 months. Naturally, curing time considerably affects the flavor. Presumably, short peptides and free amino acids play an important role in flavor development, but so do volatile compounds. Amino acids may be converted to short chain fatty acids and into thiols (H_2S and $CH_3 \cdot SH$) (see Fig. 23.5). Among the components formed via the pyruvate metabolism of the lactic acid bacteria are diacetyl, acetic acid, and ethanol, and, hence, probably also esters. CO_2 also is essential as a flavor enhancer; it is partly formed by decarboxylation of amino acids.

The fat is essential, not only for the consistency but for the flavor. Low-fat cheese has been found to lack the typical Cheddar flavor. Probably the most important role of the fat is to be a solvent for hydrophobic flavor compounds. In addition, lipolysis (if not too strong) and formation of ketones from free fatty acids play a certain part. It should be noted that the various investigators do not agree at all regarding the characteristic flavor of Cheddar.

Defects that may occur in Cheddar include the following:

- *Open texture*, which may lead to formation of cracks upon gas production during maturation. In serious cases it causes mold growth or—if the cheese is kept in dry air—autoxidation of fat (tallowy discoloration). The information given above indicates how the defect can be prevented.
- ''*Seaminess*'' refers to the appearance of whitish ''veins'' seen in a cross-section of the cheese. Most Cheddar contains small crystals of $Ca(H_2PO_4)_2$, especially when its water content is low and the salt content high. These crystals are rapidly formed if the milled curd has a high pH at salting and is heavily salted and not given enough time for adequate absorption of salt before pressing. The crystals are formed in the outermost layer of the curd pieces, causing this layer to turn white and hard. Seaminess is often accompanied by an open texture.
- *Incomplete acid production* often is responsible for insufficient flavor and abnormal consistency, and increases the risk of defects.
- *Contaminating bacteria* may cause defects, especially at high pH, low salt content, and high ripening temperature. It mostly concerns lactobacilli or pediococci that cause several off-flavors (Section 24.3). Obviously, hygiene during cheese making is essential.
- Especially in the United States, most Cheddar-type cheese is currently made in very large blocks. This makes it difficult to cool the interior of the cheese with sufficient speed; consequently, the bacterial defects just mentioned may readily occur. To prevent this, the cheeses often are kept in cold rooms, say at 5°C. It goes without saying that this also greatly impedes normal flavor development.

- A bitter flavor defect develops if the salt content is low and the curing temperature is high. Selection of nonbitter strains of lactic acid bacteria therefore is important.

25.6 CHEESES WITH A SPECIFIC FLORA

These types of cheese are characterized by their microbial ripening being controlled not only by a normal flora of lactic acid bacteria (sometimes also propionic acid bacteria), but also by either:

a. A surface flora in which *Penicillium camemberti* is the dominant organism. It mostly concerns cheeses with a soft consistency, e.g., Camembert and Brie.
b. A surface flora dominated by pigment-producing strains of coryneform bacteria such as *Arthrobacter* spp. and *Brevibacterium linens*. It concerns soft cheeses such as Pont l'Évêque, Muenster, and Herve as well as some types of hard, e.g., Gruyère, and semihard cheese, e.g., Tilsiter and Port du Salut.
c. An internal flora consisting of *Penicillium roqueforti*. Among the cheeses are the semihard Roquefort, many types designated bleu, and also Stilton, which is a harder type of cheese.

These are the most important groups. Other types are Gamalost with a blue surface mold (*Mucor* spp.), and intermediate types with *P. roqueforti* in the interior and *P. camemberti* (Lymeswold), or coryneforms (Gorgonzola) on the surface.

Below, some typical examples are discussed, i.e., soft cheese with a surface flora and cheese veined with blue mold. Characteristic aspects of cheese manufacture, factors determining the development of a specific flora, and the ensuing ripening characteristics will be considered.

25.6.1 Soft Cheese with a Surface Flora

The traditional and related manufacturing methods are characterized by:

a. Acidification of the milk prior to renneting, which yields a gel with an acidic as well as a rennet character. The intensity of preculturing determines whether a rennet gel (e.g., in the manufacture of Pont l'Évêque) or an acid gel (e.g., old-fashioned Camembert) prevails.
b. The curd is cut into large lumps that are put into molds. Hence the curd is not stirred and not washed. Syneresis largely occurs after moulding; the cheese attains a relatively high water content, usually 50% to 60%. The pH of the cheese shortly after its manufacture is low (say, 4.5–5) and the curd loses most of the calcium phosphate associ-

ated with the casein micelles and thereby much of its buffering capacity.

c. The flora growing on the cheese surface forms aroma substances and deacidifies the cheese (see Fig. 25.3). The latter factor is essential in obtaining a soft but not yet liquid consistency. Since the softening starts at the surface, the cheese loaves are made quite flat, i.e., 3–4 cm thick; otherwise, a firm and acid central portion would remain. Often, the loaf diameter is also small, e.g., 10 cm.

d. If the surface flora is allowed to grow unlimited, the mature cheese is only fit to be eaten for a short time (see also Fig. 23.10). The shelf life can be lengthened by cooling and by sterilization of canned Camembert cheese. However, the latter method does not yield a good-quality cheese.

25.6.1.1 Manufacture

Figure 25.19 gives an outline of a fairly traditional industrial manufacture of soft cheese with surface flora, made of preacidified milk.

Standardization and *pasteurization* are commonly applied. The fat content in the dry matter may range from 40% to 60%. If raw milk is used, it has to comply with the strictest bacteriological requirements because of the risk of growth of pathogenic microorganisms (e.g., *Listeria monocytogenes*), especially on the cheese surface that is deacidified during maturation.

The milk is *preacidified* by means of a homofermentative mesophilic starter. A heterofermentative starter, which produces much more CO_2, can introduce defects due to growth of microaerophilic CO_2-tolerant blue molds that may have contaminated the milk. Nevertheless, most traditional Camembert has a fairly open texture (small irregular holes). Because of the preculturing the lactic acid bacteria hardly multiply any further during cheese manufacture. The great number of cells involved enhances a rapid drop of the curd pH, causing a considerable loss of buffering compounds during the whey drainage. Thereby the curd will be demineralized to such an extent that a satisfactory consistency of the cheese can be achieved.

Furthermore, the preculturing improves the rennetability of the milk, which has been kept cool during the preacidification stage, and it causes a firmer gel, thereby reducing the loss of fines during curd making.

Various methods are available to arrive at a desirable acid production during preculturing of the milk. The inoculum percentage of the starter as well as the temperature and time of incubation of the milk can be varied. A long incubation time at low temperature requires a very good bacterial quality of the milk (e.g., the number of psychrotrophs in the raw milk should be low) and a hygienic manufacturing process to prevent contamination by homologous bacte-
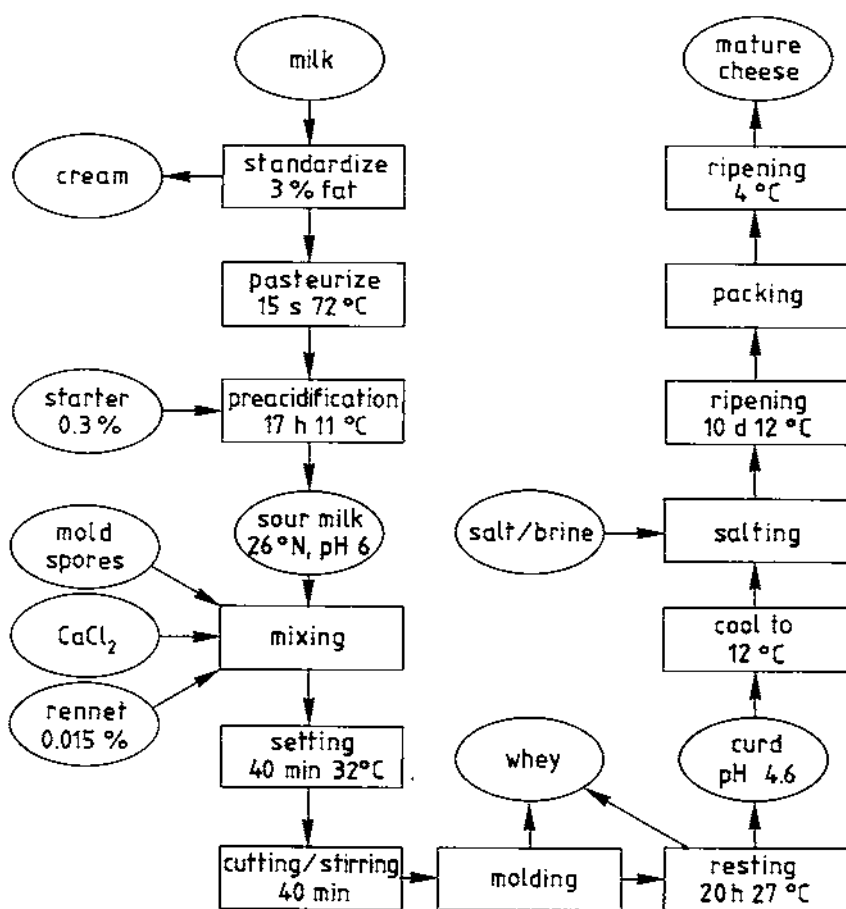
FIGURE 25.19 Example of the manufacture of soft cheese with a surface mold. Usually, the cheese is brine-salted. In the manufacture of cheese with a red smear, coryneforms instead of mold spores are added, and the cheeses are rubbed with weak brine in the first ripening stage.

riophages and growth of spoilage organisms, especially enteropathogenic coliforms.

Additions. The amount of rennet added depends on the type of cheese and the acidity of the milk. Usually, it is less than in the manufacture of harder cheeses and more than in fresh cheeses. A relatively long clotting time at high tempera-

ture, i.e., 32–34°C, contributes to the formation of a firm gel and to a further decrease of the curd pH.

Usually, $CaCl_2$ is added to the milk, and also coloring matter. At the same time the milk is inoculated with organisms that should make up the surface flora, i.e., a suspension of spores of *P. camemberti* for mold-ripened cheese and coryneform bacteria, essentially *Brevibacterium linens*, for cheese with a red smear (Rotschmiere, ''ferment du rouge''). Additional mold spores can be added to the cheese through the brine or by means of spraying in the ripening room. Coryneforms may additionally be added by means of the weak brine, used to rub the cheese surface during the ripening.

Stirring the curd, shaping. Formerly, the clotted milk was often not stirred, or the gel was only lightly agitated before shaping the cheese. The curd was put in perforated molds, their capacity largely surpassing the volume of the cheese to be made. Repeated filling up with curd as long as whey drained led to a satisfactory size of cheese. In this way, the shaping took several hours and was not suitable for mechanization. Nowadays this manufacturing protocol is still in use for farmhouse cheese, such as Camembert and Brie.

In the industrial way of manufacture, the curd is cut into pieces of, say, 2–2.5 cm. Furthermore, the curd is left in the whey for some 30–50 min to synerese; thereby a part of the whey (20% to 40%) is released and mechanical damage during continued manufacture is restricted because the curd pieces already have become rather firm. After drainage of the whey, a mass of curd needed for one loaf of cheese is put into the mold. The water content of the curd at shaping is lower than in traditionally made curd; adequate demineralization nevertheless is possible by adjusting the rate of acid production. The curd is not washed.

Syneresis and acid production during resting take a considerable time, i.e., 15–20 h. These processes are enhanced by keeping the cheese at a fairly high temperature, i.e., 26–28°C. Cooling of the cheese should be prevented (note that the specific surface area of the cheese is large). In this period the cheese is turned a few times and it sags in the mold to the desired height. The pH drops to 4.5–5; not all of the lactose is converted because the curd is not washed, and even after a considerable time some sugar is left. The Ca content of the cheese is low, about 0.4% in the dry matter (Fig. 25.4).

Salting. Nowadays the cheese is usually salted in brine. Depending on type, shape, and size of the cheese, the salting time varies from 30 min to several hours. Due to the high water content and the great specific surface area, the salt absorption is fast. After about a week the salt is evenly distributed throughout the cheese. The salt content of the cheese is about 1% to 2%.

Ripening conditions depend on whether molds or coryneform bacteria are the dominant surface flora (see also Table 22.3). Many factors affect the growth of these microorganisms (see below). The NaCl content at the cheese surface is

critical. The humidity of the air is kept higher for coryneforms than for molds. Further variations in ripening conditions are:

a. To allow *mold* growth, the cheese is kept ''untouched'' and dry. If need be, the salted cheese may be dried at a low relative humidity and a high temperature, e.g., RH 80% and 18°C, respectively. The cheese rests on a grid, allowing the mold to develop on the entire surface. Oxygen supply is essential. The air must constantly be refreshed and preferably be filtered to prevent contamination by unsiderable microorganisms. The cheese is turned every 3 or 4 days. After about 10 days it is completely enveloped by the mold and is packed. (Soft cheese with a surface flora is normally packed in perforated foil, allowing any excess of NH_3 to escape.) The maturation is not complete but continues during subsequent storage at low temperature, e.g., 4°C.

Extending the ripening period at 11–13°C with a few days causes a more intense maturation and gives a cheese with more flavor. In this time also coryneform bacteria develop, among which the orange-red *Brevibacterium linens*. Their growth is enhanced by putting the cheese, which is enveloped by a full-grown surface mold, on a nonperforated support and turning it every day. Thereby the hypha of the molds are damaged, giving the bacteria some competitive advantage. The presence of an additional bacterial flora was highly appreciated previously because of the more intense flavor obtained, but currently most manufacturers ensure that the entire cheese surface is absolutely white.

b. To allow growth of *coryneform bacteria*, the cheese is put on closed supports and is initially turned and washed every day. Washing with a salt solution or water (inoculated with the bacteria, if desired) is done mechanically. The flora is well developed after, say, 10 days and from that time on the cheese is turned and washed every 3 or 4 days. The ripening time varies from 2 to 4 weeks, depending on shape, size, and desired maturity of the cheese. These cheeses also show continued maturation during cold storage and distribution. In some varieties, the mature cheese is allowed to dry somewhat and subsequently covered with a latex coating.

The industrial manufacturing and ripening conditions of soft cheese types with a more definite rennet-gel character, largely correspond with the characteristics mentioned. Some differences in manufacture are as follows:

a. The milk is not or hardly precultured and the pH at renneting is about 6.6.
b. The renneting temperature is higher (34–36°C) and more rennet is

added, i.e., 30–40 ml per 100 L of milk. In this way a very firm coagu-
lum is obtained.

c. The gel is cut finer and more intensely stirred. More whey is drained
off before shaping.

d. Less buffering material is lost during drainage and the Ca content in
the dry matter only decreases to about 1.1% (see also Fig. 25.4). These
factors, together with the lower water content of the curd (correspond-
ing to less potential lactic acid), are responsible for a higher pH after
drainage, i.e., 4.9–5.1.

25.6.1.2 Modern Variants

New technological possibilities and economic reasons have led to alternative
types of soft cheese with a surface flora. Characteristic aspects of the manufacture
are as follows:

a. Lightly preripened milk is used.

b. The fat content in the dry matter of the cheese often is high, up to 70%.
The cheese may contain native or denatured serum proteins, either by
heating the milk or by applying ultrafiltration.

c. The thickness of the cheese loaf is enlarged as is the weight (to, say,
3 kg), and the product may be portioned and packed.

d. After shaping, the curd loses little moisture. To achieve this effect,
syneresis during curd treatment in the vat should be enhanced, espe-
cially when the composition of the curd hinders syneresis, as is true
for high-fat curd. Syneresis will suffice if much rennet is used (30–35
ml/100 L milk), the renneting temperature is high (36–39°C), the curd
is cut into small pieces (0.7–1 cm), and stirring is intensified.

e. The pH of the 1-day-old cheese is relatively high, i.e., 5–5.1, certainly
for a ''thick'' cheese. If the pH of a thick cheese would be low, its
central part cannot be deacidified by the surface flora in time. This
would lead to great differences in the ripening stage between rind and
core, as well as to an inhomogeneous consistency. Washing the curd
helps. Alternatively, the pH can be controlled by applying a thermo-
philic starter. The acid production is fast, due to the high temperature
during manufacture. Ongoing acid production is greatly hindered by
rapid cooling of the cheese after the desired pH is attained, or by brin-
ing it. This implies, of course, that the cheese still contains an apprecia-
ble amount of sugar.

Nowadays the tendency is to mature soft cheese at low temperature, e.g., 4°C.
At such a temperature the biochemical processes involved can more readily be
controlled, growth of pathogenic organisms may be prevented, and development
of a soft consistency enhanced (see also Section 23.3).

25.6.1.3 Development and Ecology of the Surface Flora

A surface flora represents a complicated ecological system. The dominant flora will depend on (a) which organisms are (initially) present and (b) how good the conditions are for growth, which—of course—varies greatly according to species and strain. The presence of microorganisms is highly dependent on any inoculation (type and number of organisms) and on contamination (thus on hygiene). Contamination of the milk can be largely undone by pasteurization because virtually all microorganisms that can grow on the surface are heat-labile.

Conditions affecting growth include temperature, pH, salt concentration, O_2 pressure, humidity of the air, availability of nutrients, and presence (and concentration) of substances that stimulate or inhibit growth. Some of these conditions may be very different at the surface as compared to those in the interior of the cheese or in the surrounding air. The conditions depend on cheese composition and on treatment (e.g., temperature, rubbing with weak brine), but also on the action of the microorganisms themselves: Nutrients may become depleted, other nutrients may be formed (e.g., amino acids from protein) to be utilized by other organisms, and inhibiting substances (e.g., antibiotics) may be excreted. The most striking change often is an increase in pH.

All of these factors determine which organisms can grow fastest and these will usually outnumber and even oust the others, unless the others were already present in very large numbers. Nevertheless, even in the latter case, the organisms will die off when conditions become very unfavorable for them. This implies that the dominant flora may change markedly during maturation.

The principles of microbial ecology of a surface flora are especially manifested in soft cheeses with low initial pH (say, 4.7). These are discussed below (see also above, Sec. 25.6.1.1).

Cheese with a Surface Mold

The predominant organisms are yeasts, micrococci, coryneform bacteria (e.g., *Brevibacterium linens*), and the molds *Geotrichum candidum* and, especially, *Penicillium camemberti. Penicillium* is inoculated, the remaining are contaminating organisms. The following are essential parameters for growth.

    a. *Temperature*. The course of the temperature during maturation was already mentioned (see also Fig. 25.1 and 25.19). The optimum temperature for growth of yeasts and molds is 20–25°C; at the ripening temperature (about 12°C) the growth rate is far slower, with *G. candidum* being most cold-sensitive. If the other conditions are favorable, micrococci and coryneform bacteria grow fastest at the ripening temperature.
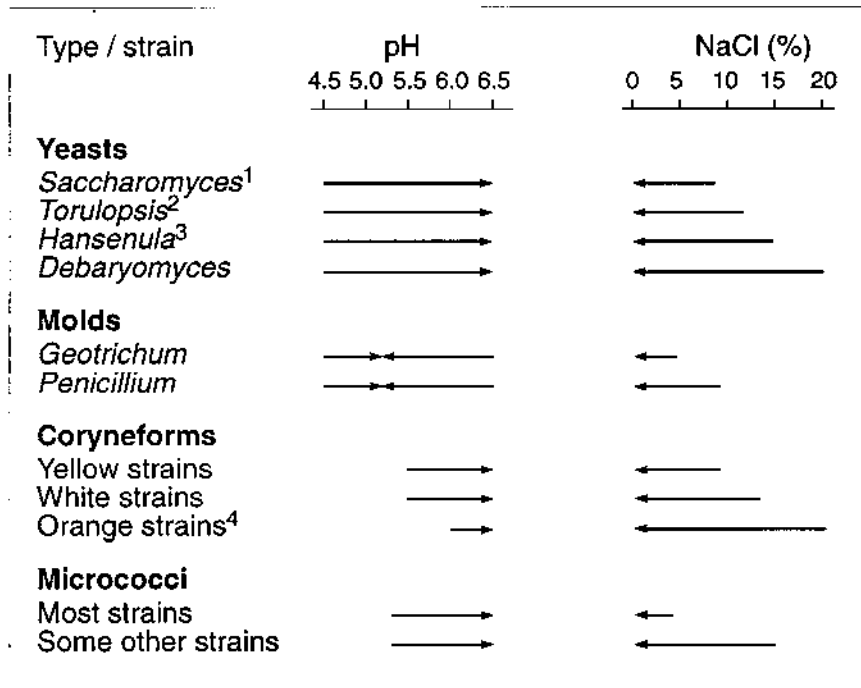
Figure 25.20   Effect of pH and salt on the possibility of some important surface organisms to grow. →, ←, increasing growth; 1, Also, e.g., *Candida*. 2, Also, e.g., *Rhodotorula*. 3, Also, e.g., *Pichia*. 4, *Brevibacterium linens*. After J. Stadhouders, *Neth. Milk Dairy J.* **29** (1975) 104.

   b. *pH and NaCl content*. Figure 25.20 shows effects on growth of organisms involved. Note that among the molds, *G. candidum* is highly salt-sensitive.

A succession of surface organisms occurs. The above three factors (temperature, pH, and salt) mainly determine the succession. The following stages can be distinguished:

   a. *Before salting*. The temperature of the cheese is high (26–28°C) and the salt content is low; the pH drops quickly, due to lactic acid production. These are favorable conditions for yeasts, which contaminate the cheese surface through air and machinery. Growth of molds is negligible in this stage because the spores have to germinate, which takes some time. Some of the yeasts ferment lactose and all of them ferment

lactic acid. A variety of yeasts can appear, depending on the actual flora in the dairy. *Saccharomyces lactis, S. fragilis, Torulopsis sphaerica*, and *Candida pseudotropicalis* are the most common species, but *Debaryomyces hansenii* or *Torulopsis candida* can also be present.

b. *After salting*. The brining causes the temperature of the cheese to decrease to about 12°C and the salt content in the rind to increase considerably. The quick salt penetration in the cheese and the fairly low equilibrium salt content in the moisture (4% to 5%) allow the yeast flora to grow further to a high final count, i.e., about 10^9 per cm^2 surface area. The pH of the cheese increases (see also Fig. 25.3) since the yeasts ferment lactic acid. This enhances the growth of the molds. Soon, *P. camemberti* overgrows *G. candidum*, the former being much less sensitive to temperature and salt. A limited growth of *G. candidum* may improve the organoleptic properties of cheese ripened with *P. camemberti* because, for example, less of a bitter flavor develops. Abundant growth, however, interferes with the growth of *P. camemberti* and causes defects, e.g., a wrinkled surface, poor flavor. After some 4 or 5 days, *Penicillium* becomes visible and after about 10 days it is full grown. The molds, including *G. candidum*, consume lactose and especially lactic acid, which causes the pH to increase faster. This increase enables the lactic acid bacteria to ferment residual lactose. At a pH above about 5.5 non-acid-tolerant micrococci and coryneforms can grow. Usually, gram-negative bacteria (e.g., *Pseudomonas* ssp.) also develop and, if present in large numbers, cause flavor defects. As mentioned, growth of pigmented coryneforms is nowadays mostly considered undesirable. Strains of *P. camemberti* have therefore been selected that have a higher optimum pH for growth and thus can suppress the coryneforms. Occasionally, uncolored strains (mutants) of *Brevibacterium linens* are added.

Cheese with a Flora of Coryneforms

As in the former group, surface organisms succeed one another. This applies to yeasts, micrococci, and especially coryneforms (added and contaminating organisms). Again, the cheese is deacidified. The growth of coryneform bacteria is greatly enhanced, whereas that of contaminating molds is suppressed. Dominance of mold growth can only occur if the mycelium can develop; any rubbing or washing destroys the hypha. The cheese surface is therefore regularly washed with an NaCl solution or with water, especially at the beginning of the maturation. Furthermore, the cheese is matured at a high relative air humidity (Table 22.3). A contiguous slimy layer forms around the cheese due to the production of microbial polysaccharides and to swelling of the protein matrix. Under these conditions, the slower growing molds lose the competition.

The flora of corynebacteria is influenced by the salt content (see Fig. 25.20). A relatively high content enhances the white and orange-red strains (*Brevibacterium linens*), a lower content the yellow strains. Note that several strains, scarcely or not pigmented in pure culture, appear red when growing on cheese. A high salt content also enhances the proteolytic activity of the bacteria. This is because they need a high concentration of proline to keep their internal osmotic pressure at a sufficiently high level compared with the environment. The proline can only be obtained by proteolysis of casein.

The above principles are also valid when a flora settles on other types of cheese, including some hard varieties. The number of yeasts involved closely depends on the initial pH of the cheese. The higher the pH, the less important the yeasts are, and the earlier a leading flora of molds or corynebacteria appears.

25.6.1.4 Consistency

During maturation, cheeses with a high water content and an initially low pH must change in consistency from fairly firm and short, through pasty, to soft and spreadable. If the cheese is left to mature for a long time it may liquefy entirely. These changes start just below the cheese rind and move inward, to the center. Formerly, enzymes from the surface flora were held responsible; penetrating into the cheese they supposedly caused a moving boundary of protein degradation. The diffusion rate of the enzymes involved is, however, far too slow; the effective diffusion coefficient of proteins in cheese is about 10^{-12} m$^2 \cdot$ s^{-1}, which implies that it would take several months for the center of the cheese to acquire a significant enzyme concentration.

Hence, the mechanism is different and the important factors are as follows (see also Section 23.6):

a. *The composition of the cheese*, in particular the water content or rather the water-to-paracasein ratio (which should be high), and the initial content of calcium phosphate (which should not be high).

b. *A sufficient protein breakdown*. Especially rennet action is important (see also Section 23.3). Maximum activity of calf rennet is at a pH of about 5.

c. *A sufficiently high pH*. At a lower pH (say, 4.7), proteolysis is still considerable but the consistency remains short and does not change until the pH is increased by the surface flora (see below). At a higher pH, plasmin and the enzymes of starter bacteria also become increasingly active, but their importance in terms of consistency is not known.

d. *The action of the surface flora*. It implies:

1. *Deacidification of the cheese*. Consumption of lactic acid causes migration of the acid to the surface and of basic protein degradation products of the flora, including NH$_3$, to the interior. This

causes a pH gradient. More NH_3 is produced at a higher (initial) pH. The pH at the surface of mature cheese soon is about 7; in the center it may increase up to 6. (The effective diffusion coefficient of low-molar mass compounds equals about 4×10^{-10} $m^2 \cdot s^{-1}$, allowing halving of a concentration difference over 1 cm in about 3 days).

2. *The removal of Ca and of phosphate*, which improves the solubility of the protein. A high pH at the surface leads to precipitation of insoluble di- and tricalcium phosphate. In turn, the precipitation causes diffusion of Ca and phosphate to the surface. In mature Camembert, about 80% of Ca and 50% of inorganic phosphate is therefore found in the outermost layer.

Naturally, an essential function of the surface flora is to produce flavor compounds (see Section 23.5). Clearly, a satisfactory consistency results from a complex of factors, i.e., cheese composition (water and salt content), sufficient protein breakdown, a fairly high pH (due to consumption of lactic acid and production of NH_3), and, presumably, a low content of Ca. Other conditions being equal and favorable, the softness increases with the NH_3 content; at a very high content, say, 0.3% NH_3, the cheese liquefies.

25.6.2 Blue-Veined Cheese

These cheeses are characterized by growth of *Penicillium roqueforti* in the interior. Many types of the cheese exist. Ewes' or cows' milk is usually used for the manufacture.

25.6.2.1 Manufacture

a. *Treatment of the milk*. Precultured raw or pasteurized milk is used. Usually, the milk is standardized. For some types of cheese, all or part of the milk is homogenized to obtain a whiter color (occasionally a whitener is added, e.g., chlorophyll), to enhance lipolysis, to improve the consistency, and to avoid creaming when the clotting time is long.

b. *Additions*

1. *Starter*. To raw milk little or no mesophilic starter is added, e.g., in the manufacture of Roquefort. To pasteurized milk a mesophilic starter is added (e.g., Stilton) or a thermophilic starter (e.g., Gorgonzola). Gas formation by leuconostocs is desirable for the mold to become satisfactorily implanted; often, these bacteria are added. They grow readily if the acid production is not too fast; because of this, little starter then is used.

2. *Spores of P. roqueforti.* Sometimes the suspension of spores is added to the curd. The selected mold can grow at low oxygen

   pressure (e.g., 5% O_2), withstands a high CO_2 content, and grows
   readily at low temperature, i.e., 5–10°C.

   3. *Rennet*. Up to 30 ml per 100 L of milk is used; the clotting temper-
      ature ranges from 30°C to 33°C and the clotting time from about
      30 min to several hours. The latter holds for Roquefort, which is
      made of ewes' milk.

c. *Curd treatment in the vat*. It is aimed at enhancing the implantation
   of the mold (see also item d). Generally, the curd is coarsely cut and
   gently stirred for a short time. The mixture of curd and whey is scalded
   and washed little or not at all.

d. *Shaping and drainage*. Often, the curd particles are slightly cooled
   before shaping, the acid production is slow (allowing the leuconostocs
   to grow significantly), the drainage takes a long time, and the curd is
   not pressed. All of these factors interfere with a quick and complete
   fusion of the curd particles. The formation of holes by the CO_2 pro-
   duced by the leuconostocs, which is facilitated by mechanical air inclu-
   sion, readily causes an open texture that enhances implantation of the
   mold. During drainage the cheese is turned from time to time, and the
   pH drops to 4.6–4.8.

e. *Salting*. Brining or dry salting is mostly applied. Roquefort is dry
   salted; Stilton is salted at the curd stage, after cheddaring.

f. *Piercing*. To enhance the mold growth, holes are pierced into the
   cheese, allowing ready penetration of air.

g. *Maturation*. The temperature is 5–10°C, the RH about 90%. During
   the period of mold growth, the surface must be kept clean to prevent
   clogging of the holes; furthermore, the piercing of holes may be re-
   peated. The ripening time ranges from, say, 3 weeks to several months.
   The pH of the mature cheese is about 6. After a time of ripening, the
   cheese is packed in metal foil (tin or aluminum) to stop the oxygen
   supply, which would cause too sharp a flavor.

25.6.2.2 Microbial Interactions

The flora of blue cheese represents a complicated ecosystem. This has been exten-
sively studied in Roquefort made from naturally contaminated raw ewes' milk
(currently starter is added). The following are some of the conclusions of those
investigations (see Fig. 25.21).

a. At the end of drainage the flora consists of:
   1. *Mesophilic lactococci: L. lactis* sspp. *lactis* and *cremoris*, about
      10^9 per gram of cheese.
   2. *Leuconostocs*, comprising over 10^6 per gram. Considerable growth
      occurs, enhanced by nitrogenous compounds formed by the more
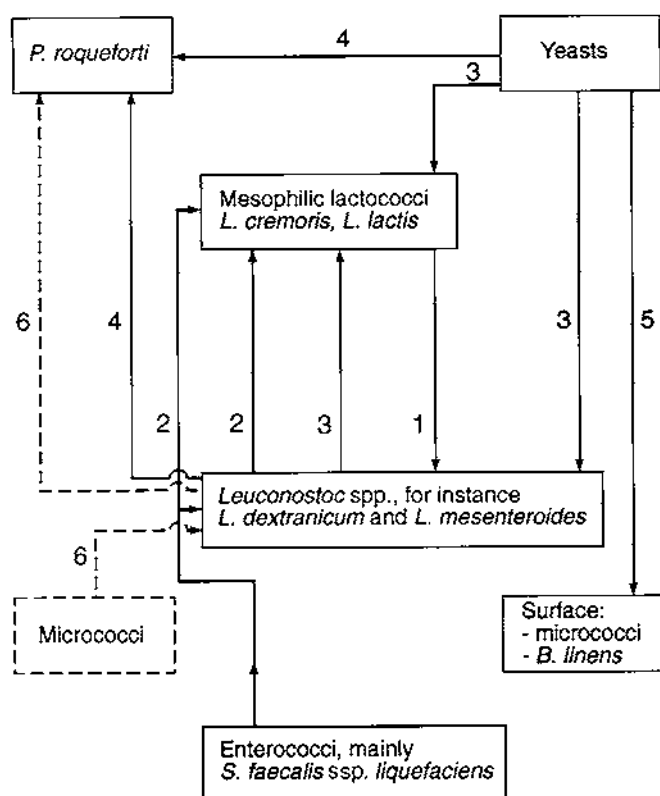
FIGURE 25.21 Microbial associations in Roquefort, made of naturally contaminated raw ewes' milk. ⟶, enhances growth; −−→, impedes growth. After Devoyod et al., *Le Lait* **52** (1972) 297.

proteolytic lactococci (number 1 in Fig. 25.21). It mainly concerns strains that do not ferment citrate and thus produce little if any CO_2 when growing in milk.

3. *Enterococci*, about 10^7 per gram. Certain species, especially *S. faecalis* ssp. *liquefaciens*, enhance the growth of and acid production by the mesophilic lactococci, and enhance the production of CO_2 by the leuconostocs; metabolites produced by the enterococci may include low-molar mass peptides and aromatic amino acids, e.g., tryptophan (number 2). CO_2 can also stimulate the mesophilic lactococci.

4. *Yeasts*, about 10^8 per gram. Before salting, it mainly concerns

lactose-fermenting salt-intolerant species, including *Saccharo-myces, Candida*, and *Torulopsis*. They likewise enhance the growth of the mesophilic lactococci and CO_2 production by leuconostocs (number 3). Due to the salting, the yeast flora shifts to lactose-not-fermenting salt-tolerant species, including *Pichia, Hansenula*, and *Debaryomyces*. (The salt content of Roquefort is high, i.e., about 10% in water.)

5. *Other organisms*, especially micrococci, staphylococci, lactobacilli, and coliforms.

CO_2 production by leuconostocs and yeasts causes holes in the cheese, creating an increased surface area for *P. roqueforti* (number 4). The mold can readily grow if air is supplied by piercing holes into the cheese.

b. Some phenomena occurring during maturation:

1. Increase of the pH at the cheese surface, mainly caused by lactic acid consumption by yeasts, affords growth possibilities for salt-tolerant aerobic micrococci and corynebacteria, especially *Brevibacterium linens* (number 5).

2. Occasional contamination of the cheese milk by strongly proteolytic micrococci can result in cheese with insufficient openness and thereby unsatisfactory mold growth. This is caused by metabolites that do not affect the growth of the leuconostocs but impede their gas production (number 6).

The microbial interactions in various blue-veined cheeses will roughly correspond to those found in Roquefort. Types of cheese will vary, however, according to composition and extent of contamination of the raw milk, heat treatment of the milk, recontamination, way of manufacture of the cheese, and ripening conditions. To ensure that a satisfactory open texture is achieved, citrate fermenting, CO_2 producing *Leuconostoc cremoris* bacteria are now commonly added to the cheese milk.

The specific flora of blue cheese largely determines the organoleptic quality of that cheese (see Section 23.5). Moreover, variations in manufacturing process and kind of milk affect cheese quality. For instance, blue-veined cheeses made from cows' and ewes' milk differ considerably in flavor.

25.7 PROCESSED CHEESE

Some varieties of cheese have a long shelf life, but other varieties, mainly high-moisture cheeses, do not. Therefore, attempts have been made to preserve cheese. Flat pieces of cheese or curd can be left to dry in the sun. Alternatively, the cheese can be preserved by smoking, as is applied for some Italian cheeses, e.g.,

caciocavallo. Cheese can be stored in brine (feta, Domiati) or even be boiled in brine. Small balls of (fresh) cheese can be candied. Pasteurization of cheese can also be tried, but that generally leads to melting of the cheese, followed by demixing: Oil separates and the proteinaceous mass may become lumpy or a kind of serum may separate. Such demixing can be prevented by adding certain salts. In this way processed cheese evolved.

Processed cheese is made by grinding and blending cheese, adding ''emulsifying salts,'' heating while stirring to, say, 80°C, stirring the melted mass for several minutes at that temperature, putting it into suitable containers, and cooling. The cooling causes setting of the mass. Continuous processing in scraped-surface heat exchangers or by steam injection heating is also applied. In addition to cheese, water, butterfat, whey powder, and/or caseinate is often added. The flavor of processed cheese distinctly differs from that of cheese, partly due to the heat treatment, partly to the melting salts. Various additional ingredients, such as ham, celery, or nuts, are therefore sometimes added.

The main function of the melting salts is to bind Ca without the formation of a precipitate. Citrate and polyphosphate (preferably a long chain polyphosphate) are especially suitable. Due to the binding of Ca, the protein mass remains homogeneous and tends to swell. This especially is needed to keep the fat in an emulsified state because the fat globules tend to readily coalesce during melting. Still other reactions occur, but these are poorly understood. The protein mass tends to aggregate (coagulate) after stirring for a while at high temperature. Addition of a small amount of processed cheese—the remainder of a previous batch— as well as the use of well-ripened cheese enhances this coagulation. Processed cheese cannot be made of well-ripened cheese. An excess of hardly ripened cheese is commonly used, and some mature cheese is added for flavor.

Two groups of processed cheese can be distinguished, spreads and sliceable blocks. The former contains, say, 58% water and 50% fat in the dry matter; citrate cannot be applied because it causes a firm cheese mass. For instance, the sliceable cheese contains 46% water. The quantity of melting salt required amounts to 10% (or even more) of the quantity of protein, which leads to 2% to 3% in the cheese.

Growth of clostridia may spoil the product. Nisin can be added to prevent their growth. Presently the melting operation may be continuous and a kind of ultrahigh-temperature treatment be used to kill the spores.

Formerly, the processed cheese manufacture was specifically meant to enhance the market value of cheese of unsatisfactory quality. The blending of several lots, including cheese of satisfactory quality, resulted in an acceptable product. Processed cheese is now being made as a product with specific desirable properties, having a long shelf life, being homogeneous, easy to handle, with several ingredients added. In some countries (e.g., the United States and Germany) it makes up an essential part of the cheese market.

A related product is ''Kochkäse'' (Germany) or ''Cancaillotte'' (France). One starts from low-fat quarg of pH about 4.5 that is ripened for a few days at 23–30°C in a layer of 5–10 cm thickness. The layer is turned regularly to incorporate oxygen. The air humidity should be high to prevent moisture loss. Yeasts are now growing and consume lactate, raising the pH to 5.6–6. The yeasts involved will be similar to those in a developing red smear (see Section 25.6.1) and also coryneform bacteria may develop. The product so obtained allows subsequent melting without the addition of melting salts because the acidic quarg has a low content of Ca. The ripened curd, 1% to 2% salt, a fair amount of water or milk, and butter are blended and melted while being stirred for, say, 10 min at 90°C. Nowadays a higher heating temperature is often applied, up to 115°C. The liquid mass is placed in cups and cooled. It is consumed as a smooth, light paste.

SUGGESTED LITERATURE

- A comprehensive survey of cheese varieties is given in:
    G. Burkhalter (Rapporteur), IDF Catalogue of Cheeses, IDF Bulletin, Document 141, 1981.
- Several cheese varieties are dealt with, generally in great depth, in:
    P. F. Fox, ed. *Cheese: Chemistry, Physics and Microbiology, Vol. 2, Major Cheese Groups*, 2nd ed., Chapman and Hall, London, 1993.
- Valuable information can be found in the encyclopedic part of the book:
    H. Mair-Waldburg, *Handbuch der Käse*, Volkswirtschaftlicher Verlag GmbH, Kempten, 1974.
- Much information about fresh cheese is also given in:
    F. V. Kosikowski and V. V. Mistry, *Cheese and Fermented Milk Foods*, 3rd ed., two volumes, published by the authors, 1997.
- Practical information concerning cottage cheese can be obtained from:
    D. B. Emmons and S. L. Tuckey, *Cottage Cheese and Other Cultured Milk Products*, Chas. Pfizer and Co., New York, 1967.

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