10 Milk and Dairy Products

10.1 Milk

Milk is the secreted fluid of the mammary glands of female mammals. It contains nearly all the nutrients necessary to sustain life. Since the earliest times, mankind has used the milk of goats, sheep and cows as food. Today the term "milk" is synonymous with cow's milk. The milk of other animals is spelled out, e. g., sheep milk or goat milk, when supplied commercially.

In Germany, the yield of milk per cow in kg/year has increased steadily as a result of selective breeding and improvements in feed. The yield was 1260 kg per cow in 1812, 2163 kg in 1926, 3800 kg in the FRG in 1970, 4181 kg in 1977 and 6537 kg in 2003. In the EU in 2003, Swedish cows were the best performers at 8073 kg, followed by Danish and Dutch animals at 7889 kg and 7494 kg respectively.

In some countries it is permitted to increase the yield of milk by injection of the growth hormone bovine somatropin (BST). The recombinant BST (rBST) used is identical in activity to natural BST. This is done by taking, from the DNA of cows, the specific gene sequence that carries the instructions for preparing BST and inserting it into *E. coli*, which can then produce large amounts of rBST. Natural BST consists of 190 or 191 amino acids. rBST may differ slightly in that a few extra amino acids may be attached at the N-terminal end of the BST molecule. Due to differences in the molecular mass it is possible to distinguish between rBST and natural BST. Milk production in various countries, its processing into dairy products and its consumption are summarized in Tables 10.1–10.3.

10.1.1 Physical and Physico-Chemical Properties

Milk is a white or yellow-white, opaque liquid. The color is influenced by scattering and absorption of light by milk fat globules and protein micelles. Therefore, skim milk also retains its white color. A yellowish, i. e. yellow-green, color is derived from carotene (ingested primarily during pasture grazing) present in the fat phase and from riboflavin present in the aqueous phase. Milk tastes mildly sweet, while its odor and flavor are normally quite faint.

Milk fat occurs in the form of droplets or globules, surrounded by a membrane and emulsified in milk serum (also called whey). The fat globules (called cream) separate after prolonged storage or after centrifugation. The fat globules float on the skim milk. Homogenization of milk so finely divides and emulsifies the fat globules that cream separation does not occur even after prolonged standing.

Proteins of various sizes are dispersed in milk serum. They are called micelles and consist mostly of calcium salts of casein molecules. Furthermore, milk contains lipoprotein particles, also called milk microsomes, which consist of the residues of cell membranes, microvilli, etc., as well as somatic cells, which are mainly leucocytes (10^8/l of milk). Some of the properties of the main structural elements of milk are listed in Table 10.4.

Various proteins, carbohydrates, minerals and other ingredients are solubilized in milk serum. The specific density of milk decreases with increasing fat content, and increases with increasing amounts of protein, milk sugar and salts. The specific density of cow's milk ranges from 1.029 to 1.039 (15 °C). Defatted (skim) milk has a higher specific density than whole milk. From the relationships given by *Fleischmann*:

$$m = 1.2f + \frac{266.5(s-1)}{s} \tag{10.1}$$

and by *Richmond*:

$$m = 0.25s + 1.21f + 0.66 \tag{10.2}$$

Table 10.1. Production of milk, 2006 (1000 t)

Continent	Cow milk	Buffalo milk	Sheep milk	Goat milk
World	549,693	80,094	8723	13,801
Africa	24,674	2300	1719	3129
America, Central-	14,179	–	–	–
America, North-	90,564	–	–	–
America, South- and Caribbean	66,030	–	36	164
Asia	134,170	77,571	4006	7821
Europe	209,441	222	2963	2479
Oceania	24,814	–	–	–

Country	Cow milk
USA	82,463
India	39,775
China	32,249
Russian Fed.	31,074
Germany	28,453
Brazil	25,333
France	24,195
UK	14,577
New Zealand	14,498
Ukraine	12,988
Poland	11,982
Italy	11,013
Netherlands	10,532
Australia	10,250
Mexico	10,029
Turkey	10,026
Pakistan	9404
Japan	8134
Argentina	8100
Canada	8100
Colombia	6770
∑ (%)[a]	75

Country	Buffalo milk
India	52,100
Pakistan	21,136
China	2850
Egypt	2300
Nepal	927
Iran	232
Italy	215
Myanmar	171
Turkey	38
Viet Nam	31
∑ (%)[a]	100

Country	Sheep milk
China	1091
Turkey	790
Greece	752
Syria	604
Italy	554
Romania	545
Iran	534
Sudan	487
Spain	403
France	263
Algeria	210
Mali	128
Bulgaria	108
Portugal	100
∑ (%)[a]	75

Country	Goat milk
India	3790
Sudan	1519
Bangladesh	1416
Pakistan	676
France	583
Greece	511
Spain	423
Iran	365
China	262
Ukraine	258
Russian Fed.	256
Turkey	254
∑ (%)[a]	75

[a] World production = 100%.

Table 10.2. Production of dairy products in 2004 (1000 t)

Continent	Cheese	Butter[a]	Condensed milk	Whole milk powder	Skim milk powder[b]	Whey powder
World	17,824	7968	3892	2702	3455	2038
Africa	915	226	64	21	11	2
America, North-, Central-	4944	646	1112	140	796	542
America, South-	668	191	377	768	64	–
Asia	1090	3678	559	83	239	4
Europe	9558	2622	1760	946	1699	1386
Oceania	649	605	21	744	647	105

Country	Cheese	Country	Butter[a]	Country	Condensed milk
USA	4357	India	2500	USA	797
Germany	1852	Pakistan	557	Germany	505
France	1840	USA	525	The Netherlands	291
Italy	1320	New Zealand	473	Peru	274
The Netherlands	670	Germany	440	Russian Fed.	193
Egypt	661	France	420	Thailand	179
Poland	520	Russian Fed.	262	Malaysia	164
Russian Fed.	483	Poland	180	Mexico	158
UK	370	UK	160	UK	139
Australia	364	Iran	150	China	114
Argentina	360	Ireland	142	Ukraine	80
Canada	360	Australia	130	Canada	78
Denmark	335	Italy	125	∑ (%)[c]	76
∑ (%)[c]	76	∑ (%)[c]	76		

Country	Whole milk powder	Country	Skim milk powder[b]	Country	Whey powder
New Zealand	557	USA	674	France	610
Brazil	420	New Zealand	425	USA	493
France	220	France	271	Germany	262
Australia	187	Germany	250	The Netherlands	219
Argentina	165	Russian Fed.	243	Australia	82
The Netherlands	112	Australia	222	UK	56
Mexico	105	Japan	180	Canada	49
UK	90	Poland	140	Denmark	39
Russian Fed.	85	Ukraine	117	Finnland	32
Denmark	80	Canada	102	Ireland	30
∑ (%)[c]	75	∑ (%)[c]	76	∑(%)[c]	92

[a] Including fat from buffalo milk (ghee)
[b] Including butter-milk powder
[c] World production = 100%

the dry matter content of milk, m, in percent, can be calculated from the percent fat content (f), knowing the specific density (s).

The freezing point of milk is −0.53 to −0.55 °C. This rather constant value is a suitable test for detection of watering of milk.

The pH of fresh milk is 6.5–6.75, while the acid degree according to *Soxhlet–Henkel* (°SH) is 6.5–7.5.

The refractive index (n_D^{20}) is 1.3410–1.3480, and the specific conductivity at 25 °C is 4–5.5×10^{-3} $ohm^{-1}cm^{-1}$.

Table 10.3. Consumption of milk and dairy products in FR Germany (in kg/capita and year)

	1996	2003	2005
Consumer milk	66.7	66	67
Fresh milk products (without yoghurt)	9.9	12.2	12
Yoghurt	13.1	15.3	16.8
Cream and cream products	7.6	7.4	7.4
Butter	7.3	6.6	6.5

The measurement of redox potentials of milk and its products can also be of value. The redox potential is +0.30 V for raw and +0.10 V for pasteurized milk, +0.05 V for processed cheese, −0.15 V for yoghurt and −0.30 V for Emmental cheese.

Table 10.5. Composition of human milk and milk of various mammals (%)

Milk	Protein	Casein	Whey protein	Sugar	Fat	Ash
Human	0.9[a]	0.4	0.5	7.1	4.5	0.2
Cow (bovine)	3.2	2.6	0.6	4.6	3.9	0.7
Donkey	2.0	1.0	1.0	7.4	1.4	0.5
Horse	2.5	1.3	1.2	6.2	1.9	0.5
Camel	3.6	2.7	0.9	5.0	4.0	0.8
Zebu	3.2	2.6	0.6	4.7	4.7	0.7
Yak	5.8			4.6	6.5	0.9
Buffalo	3.8	3.2	0.6	4.8	7.4	0.8
Goat	3.2	2.6	0.6	4.3	4.5	0.8
Sheep	4.6	3.9	0.7	4.8	7.2	0.9
Reindeer	10.1	8.6	1.5	2.8	18.0	1.5
Cat	7.0	3.8	3.2	4.8	4.8	0.6
Dog	7.4	4.8	2.6			
Rabbit	10.4					

[a] After the 15-th day of the breast feeding period the protein content is increased to 1.6%.

10.1.2 Composition

The composition of dairy cattle milk varies to a fairly significant extent. Table 10.5 provides some data. In all cases water is the main ingredient of milk at 63–87%. In the following sections, only cow's milk will be dealt with in detail since it is the main source of our dairy foods.

10.1.2.1 Proteins

In 1877 *O. Hammarsten* distinguished three proteins in milk: casein, lactalbumin and lactoglobulin. He also outlined a procedure for their separation: skim milk is diluted then acidified with acetic acid. Casein flocculates, while the whey proteins stay in solution. This established a specific property of casein: it is insoluble in weakly acidic media. It was later revealed that the milk protein system is much more complex. In 1936 *Pedersen* used ultra-centrifugation to demonstrate the nonhomogeneity of casein, while in 1939 *Mellander* used electrophoresis to prove that casein consists of three fractions, i. e. α-, β- and γ-casein. The most important proteins of milk are listed in Table 10.7. The casein fraction forms the main portion. Major constituents of whey proteins, β-lactoglobulin A and B and α-lactalbumin, can be differentiated genetically. Other protein constituents, e. g., enzymes, are present in much lower quantities; they are not listed in Table 10.7.

Table 10.4. Main structural elements of milk

Name	Type of dispersion	Percentage	Number (1^{-1})	Diameter (mm)	Surface (m^2/l milk)	Specific density[a] (g/ml)
Fat globules	Emulsion	3.8	10^{13}	100–10,000	70	0.92
Casein micelles	Suspension	2.8	10^{17}	10–300	4000	1.11
Globular proteins (whey proteins)	Colloidal solution	0.6	10^{20}	3–6	5000	1.34
Lipoprotein particles	Colloidal suspension	0.01	10^{17}	10	10	1.10

[a] 20 °C.

Table 10.6. Amino acid composition (g AA/100 g protein) of the total protein, casein, and whey protein of bovine milk

Amino acid	Total protein	Casein	Whey protein
Alanine	3.7	3.1	5.5
Arginine	3.6	4.1	3.3
Aspartic acid	8.2	7.0	11.0
Cystine	0.8	0.3	3.0
Glutamic acid	22.8	23.4	15.5
Glycine	2.2	2.1	3.5
Histidine	2.8	3.0	2.4
Isoleucine	6.2	5.7	7.0
Leucine	10.4	10.5	11.8
Lysine	8.3	8.2	9.6
Methionine	2.9	3.0	2.4
Phenylalanine	5.3	5.1	4.2
Proline	10.2	12.0	4.4
Serine	5.8	5.5	5.5
Threonine	4.8	4.4	8.5
Tryptophan	1.5	1.5	2.1
Tyrosine	5.4	6.1	4.2
Valine	6.8	7.0	7.5

The amino acid composition of the total protein, casein, and whey protein of bovine milk is presented in Table 10.6.

10.1.2.1.1 Casein Fractions

The main constituents of this milk protein fraction have been fairly well investigated.
Their amino acid sequences are summarized in Table 10.8. Data showing the genetic variations are provided in Table 10.9. Caseins are not denaturable because of the lacking tertiary structure.

α_s-Caseins. The B variant of α_{s1}-casein consists of a peptide chain with 199 amino acid residues and has a molecular weight of 23 kdal. The sequence contains 8 phosphoserine residues, 7 of which are localized in positions 43–80, and these positions have an additional 12 carboxyl groups. Thus these positions are extremely polar acidic segments along the peptide chain. Proline is uni-

Table 10.7. Bovine milk proteins

Fraction	Genetic variants	Portion[a]	Isoionic point	Molecular weight[b] (kdal)	Phosphorus content (%)
Caseins		80	–	–	0.9
α_{s1}-Casein	A, B, C, D, E	34	4.92–5.35	23.6[f]	1.1
α_{s2}-Casein	A, B, C, D	8		25.2[g]	1.4
κ-Casein	A, B, C, E	9	5.77–6.07	19[h]	0.2
β-Casein	A^1, A^2, A^3, B, C, D, E	25	5.20–5.85	24	0.6
γ-Casein		4	5.8–6.0	12–21	0.1
γ_1-Casein	A^1, A^2, A^3, B			20.5	
γ_2-Casein	A^1/A^2, A^3, B			11.8	
γ_3-Casein	$A^1/A^2/A^3$, B			11.6	
Whey proteins		20	–	–	
β-Lactoglobulin	A, B, C, D, E, F, G	9	5.35–5.41	18.3	
α-Lactalbumin	A, B, C	4	4.2–4.5[e]	14.2	
Serum albumin	A	1	5.13	66.3	
Immunoglobulin		2			
IgG1			5.5–6.8	162	
IgG2			7.5–8.3	152	
IgA			–	400[c]	
IgM			–	950[d]	
FSC(s)[i]				80	
Proteose-Peptone		4	3.3–3.7	4–41	

[a] As % of skim milk total protein, [b] monomers, [c] dimer, [d] pentamer, [e] isoelectric point, [f] Variant B, [g] Variant A, [h] Variant A^2, [i] Free secretory component

Table 10.8. Amino acid sequences of bovine milk proteins

α_{s1}-*Casein B-8P*																		
R	P	K	H	P	I	K	H	Q	G	L	P	Q	E	V	L	N	E	N	L
L	R	F	F	V	A	P	F	P	Q	V	F	G	K	E	K	V	N	Q	L
S	K	D	I	G	S^a	E	S^a	T	E	D	Q	A	M	E	D	I	K	E	M
E	A	E	S^a	I	S^a	S^a	S^a	E	E	I	V	P	N	S^a	V	Q	E	K	H
I	Q	K	E	D	V	P	S	E	R	Y	L	G	Y	L	E	Q	L	L	R
L	K	K	Y	K	V	P	Q	L	E	I	V	P	N	S^a	A	E	E	R	L
H	S	M	K	E	G	I	H	A	Q	Q	K	E	P	M	I	G	V	N	Q
E	L	A	Y	F	Y	P	E	L	F	R	Q	F	Y	Q	L	D	A	Y	P
S	G	A	W	Y	Y	V	P	L	G	T	Q	Y	T	D	A	P	S	F	S
D	I	P	N	P	I	G	S	E	N	S	E	K	T	T	M	P	L	W	
α_{s2}-*Casein A-11P*																		
K	N	T	M	E	H	V	S^a	S^a	S^a	E	E	S	I	I	S^a	Q	E	T	Y
K	Q	E	K	N	M	A	I	N	P	S	K	E	N	L	C	S	T	F	C
K	E	V	V	R	N	A	N	E	E	E	Y	S	I	G	S^a	S^a	S^a	E	E
S^a	A	E	V	A	T	E	E	V	K	I	T	V	D	D	K	H	Y	Q	K
A	L	N	E	I	N	E	F	Y	Q	K	F	P	Q	Y	L	Q	Y	L	Y
Q	G	P	I	V	L	N	P	W	D	Q	V	K	R	N	A	V	P	I	T
P	T	L	N	R	E	Q	L	S^a	T	S^a	E	E	N	S	K	K	T	V	D
M	E	S^a	T	E	V	F	T	K	K	T	K	L	T	E	E	E	K	N	R
L	N	F	L	K	K	I	S	Q	R	Y	Q	K	F	A	L	P	Q	Y	L
K	T	V	Y	Q	H	Q	K	A	M	K	P	W	I	Q	P	K	T	K	V
I	P	Y	V	R	Y	L													
β-*Casein* A^2-*5P*																		
R	E	L	E	E	L	N	V	P	G	E	I	V	E	S^a	L	S^a	S^a	S^a	E
E	S	I	T	R	I	N	K	K	I	E	K	F	Q	S^a	E	E	Q	Q	Q
T	E	D	E	L	Q	D	K	I	H	P	F	A	Q	T	Q	S	L	V	Y
P	F	P	G	P	I	P	N	S	L	P	Q	N	I	P	P	L	T	Q	T
P	V	V	V	P	P	F	L	Q	P	E	V	M	G	V	S	K	V	K	E
A	M	A	P	K	H	K	E	M	P	F	P	K	Y	P	V	Q	P	F	T
E	S	Q	S	L	T	L	T	D	V	E	N	L	H	L	P	P	L	L	L
Q	S	W	M	H	Q	P	H	Q	P	L	P	P	T	V	M	F	P	P	Q
S	V	L	S	L	S	Q	S	K	V	L	P	V	P	E	K	A	V	P	Y
P	Q	R	D	M	P	I	Q	A	F	L	L	Y	Q	Q	P	V	L	G	P
V	R	G	P	F	P	I	I	V											
κ-*Casein B-1P*																		
Z^d	E	Q	N	Q	E	Q	P	I	R	C	E	K	D	E	R	F	F	S	D
K	I	A	K	Y	I	P	I	Q	Y	V	L	S	R	Y	P	S	Y	G	L
N	Y	Y	Q	Q	K	P	V	A	L	I	N	N	Q	F	L	P	Y	P	Y
Y	A	K	P	A	A	V	R	S	P	A	Q	I	L	Q	W	Q	V	L	S
D	T	V	P	A	K	S	C	Q	A	Q	P	T	T	M	A	R	H	P	H
P	H	L	S	F	M	A	I	P	P	K	K	N	Q	D	K	T	E	I	P
T	I	N	T	I	A	S	G	E	P	T^b	S	T^b	P	T^b	I	E	A	V	E
S	T	V	A	T	L	E	A	S^a	P	E	V	I	E	S	P	P	E	I	N
T	V	Q	V	T	S	T	A	V											

formly distributed along the chain and apparently to a great extent hinders the formation of a regular structure. A portion of the chain, up to 30%, is assumed to have regular conformations. Amino acid residues 100–199 are distinctly apolar and are responsible for strong association tendencies,

Table 10.8. continued

α-Lactalbumin B[c]																			
E	Q	L	T	K	C	E	V	F	R	E	L	K	D	L	K	G	Y	G	G
V	S	L	P	E	W	V	C	T	T	F	H	T	S	G	Y	D	T	E	A
I	V	E	N	N	Q	S	T	D	Y	G	L	F	Q	I	N	N	K	I	W
C	K	N	D	Q	D	P	H	S	S	N	I	C	N	I	S	C	D	K	F
L	N	N	D	L	T	N	N	I	M	C	V	K	K	I	L	D	K	V	G
I	N	Y	W	L	A	H	K	A	L	C	S	E	K	L	D	Q	W	L	C
E	K	L																	
β-Lactoglobulin B[e]																			
L	I	V	T	Q	T	M	K	G	L	D	I	Q	K	V	A	G	T	W	Y
S	L	A	M	A	A	S	D	I	S	L	L	D	A	Q	S	A	P	L	R
V	Y	V	E	E	L	K	P	T	P	E	G	D	L	E	I	L	L	Q	K
W	E	N	G	E	C	A	Q	K	K	I	I	A	E	K	T	K	I	P	A
V	F	K	I	D	A	L	N	E	N	K	V	L	V	L	D	T	D	Y	K
K	Y	L	L	F	C	M	E	N	S	A	E	P	E	Q	S	L	A	C	Q
C	L	V	R	T	P	E	V	D	D	E	A	L	E	K	F	D	K	A	L
K	A	L	P	M	H	I	R	L	S	F	N	P	T	Q	L	E	E	Q	C
H	I																		

[a] The serine residue is phosphorylated.
[b] These threonine residues can be glycosylated.
[c] Disulfide bonds: 6–120, 28–111, 61–77, 73–91.
[d] Pyrrolidone carboxylic acid.
[e] Disulfide bonds: 66–160 and apparently either 106–119 or 106–121. Accordingly, the free thiol group is either Cys-119 or Cys-121.

which are limited by the repulsing forces of phosphate groups. In the presence of Ca^{2+} ions, in the levels found in milk, α_{s1}-casein forms an insoluble Ca-salt. In the A variant of the molecule, amino acid residues 14–26 are missing; in the C variant the glutamic acid in position 192 (Glu-192) is replaced by Gly-192; and in the D variant Pth-53 (phosphothreonine) replaces Ala-53.

α_{s2}-Casein (M_r 25,000) consists of 207 amino acid residues, has a pronounced dipolar structure with a concentration of anionic groups in the region of the N-terminus and cationic groups in the region of the C-terminus. It contains 11 phosphoserine and 2 cysteine residues and is even more easily precipitable with $Ca^{2\oplus}$ than α_{s1}-casein. Other proteins, previously known as α_{s3}-, α_{s4}-, α_{s5}-, and α_{s6}-caseins, appear to be members of the α_{s2} family and to differ in the degree of phosphorylation. Dimers linked via disulfide bridges also appear to be present.

β-Caseins. The A^2 variant is a peptide chain consisting of 209 residues and has a molecular weight of 24.0 kdal. Five phosphoserine residues are localized in positions 1–40; these positions contain practically all of the ionizing sites of the molecule. Positions 136–209 contain mainly residues with apolar side chains. On the whole, β-casein is the most hydrophobic casein. The molecule has a structure with a "polar head" and an "apolar tail", thus resembling a "soaplike" molecule. Indeed, CD measurements have shown that β-casein contains about 9% of α-helix structure and about 25% of β-structure. An increase in temperature results in an increase in the β-structure at the cost of the aperiodic part. The self-association of β-casein is an endothermic process. Like α_{s1}-casein, β-casein contains no cysteine. The protein precipitates in the presence of Ca^{2+} ions at the levels found in milk. However, at temperatures at or below 1 °C the calcium salt is quite soluble.

κ-Caseins. The B variant consists of a peptide chain with 169 residues and has a molecular weight of 18 kdal. The monomer, which contains 1 phosphoserine and 2 cysteine residues, is accessible only under reducing conditions.

Table 10.9. Amino acid sequences[a] of genetic variants of bovine milk proteins

Protein	Variant	Frequency[b]	Positions of the substitutions							
			14–26		53		59		192	
α_{s1}-Casein	A	s	are lacking							
(199 AS)	B	w			Ala		Glu		Glu	
	C	i							Gly	
	D	s			ThrP					
	E	s					Lys		Gly	
α_{s2}-Casein			33	47	50–58	130				
(207 AS)	A		Glu	Ala		Thr				
	B									
	C		Gly	Thr		Ile				
	D				lacking					
			18	35	36	37	67	106	122	
β-Casein	A^1						His			
(209 AS)	A^2	w, i	SerP	SerP	Glu	Glu	Pro	His	Ser	
	A^3							Gln		
	B	s					His		Arg	
	C	s		Ser		Lys	His			
	D	s	Lys							
	E	s				Lys				
κ-Casein				97	136	148	155			
(169 AS)	A	x			Thr	Asp	Ser			
	B	w, i		Arg	Ile	Ala				
	C				His					
	E						Gly			
			10							
α-Lactalbumin	A	i	Gln							
(123 AS)	B	w	Arg							
β-Lactoglobulin			45	50	59	64	78	118	130	158
(162 AS)	A	x				Asp		Val		
	B	w, i	Glu	Pro	Gln	Gly	Ile	Ala	Asp	Gln
	C				His					
	D		Gln							
	E									Gly
	F			Ser					Tyr	Gly
	G						Met			Gly

[a] cf. Table 10.8.

[b] w: predominant in the western world (*Bos taurus*), i: predominant in India (*Bos indicus, Bos grunniens*), s: rare, x: not predominant, but not rare.

Normally, κ-casein occurs as a trimer or as a higher oligomer in which the formation of disulfide bonds is probably involved. The protein contains varying amounts of carbohydrates (average values: 1% galactose, 1.2% galactosamine, 2.4% N-acetyl neuramic acid) that are bound to the peptide chain through Thr-131, 133, 135 or (in variant A) 136. κ-Casein is separated electrophoretically into various components that have the same composition of amino acids, but differ in their carbohydrate moiety, e. g., per protein molecule they contain 0–3 moles N-acetyl neuramic acid, 0–4 moles galatose and 0–3 moles galactosamine. Three different glycosyl residues could be isolated, one of which has the structure shown in Formula 10.3.

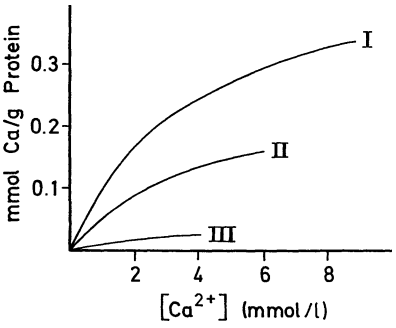


Fig. 10.1. Calcium binding by I: α_{s1}-casein (0.38), II: β-casein (0.21) and III: κ-casein (0.05). The bound phosphate residues in mmol/g of casein are given in brackets (according to *Walstra* and *Jenness*, 1984)

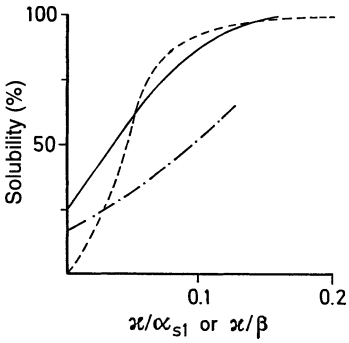
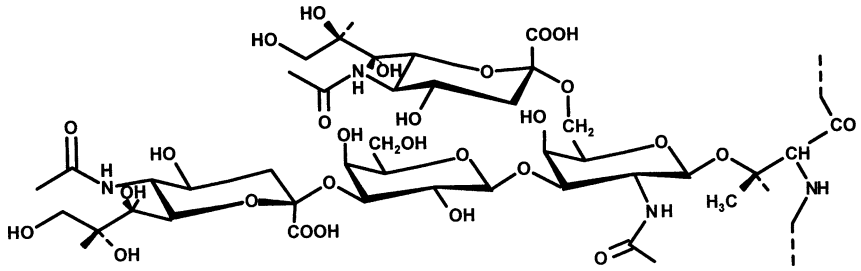


Fig. 10.2. Influence of κ-casein on the solubility of κ_{s1}-casein. (−2.5 mg/ml) and β-casein (−1.5 mg/ml; −6 mg/ml) at pH 7.0, 30 °C, 100 mmol/l $CaCl_2$ (according to *Walstra* and *Jenness*, 1984)

In the other two oligosaccharide units, one of the two N-acetylneuraminic acid residues is lacking in each case.

κ-Casein is the only main constituent of casein which remains soluble in the presence of Ca^{2+} ions in the concentrations found in milk (Fig. 10.1). Aggregation of α_{s1}- and β-caseins with κ-casein prevents their coagulation in the presence of Ca^{2+} ions (Fig. 10.2). This property of κ-casein is of utmost importance for formation and maintenance of stable casein complexes and casein micelles, as occur in milk. Chymosin (rennet, rennin cf. 1.4.5.2) selectively cleaves the peptide chain of κ-casein at $-Phe^{105} - Met^{106}-$ into two fragments: para-κ-casein and a glycopeptide (Pyg = pyroglutamic acid, i. e. pyrrolidone carboxylic acid):

The released glycopeptide is soluble, while para-κ-casein precipitates in the presence of Ca^{2+} ions. In this way κ-casein loses its protective effect; the casein complexes and casein micelles coagulate (curdle formation) from the milk. The specificity of rennin is high, as is shown in Table 10.10. If Met^{106} in κ-casein is replaced with Phe^{106} by genetic engineering techniques, the rate of catalysis is increased by 80%. The sugar moiety of κ-casein is not essential for rennin action, nor for the stabilizing property of its protein portion. However, the sugar moiety delays protein cleavage by rennin. Also, it appears that the stability of α_s- and κ-casein mixtures in the presence of Ca^{2+} ions is influenced by the carbohydrate content of κ-casein.



(10.3)

	1	105	106	169
	Pyg ·····	Phe	− Met	····· Val
		κ-Casein		
	1	105	106	169
Lab ⟶	Pyg ·····	Phe	+ Met	····· Val
	para-κ-Casein		Glycopeptide	

(10.4)

Table 10.10. Chymosin specificity: relative rate of hydrolysis of peptides from the κ-casein amino acid sequence

Substrate	V^a_{rel}
105 106 Phe-Met	0.00
104 108 Ser-Phe-Met-Ala-Ile	0.04
109 Ser-Phe-Met-Ala-Ile-Pro	0.11
103 Leu-Ser-Phe-Met-Ala-Ile	21.6
102 His-Leu-Ser-Phe-Met-Ala-Ile	31
110 Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro	100
101 Pro-His-Leu-Ser-Phe-Met-Ala-Ile	100
98 112 His-Pro-His-Pro-His-Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-Lys	2500

[a] Relative rate: $kcat/K_m$.

In the C variant of κ-casein, Arg^{97} is replaced with His^{97} (Table 10.9), which has a weaker positive charge. As a result, chymosin is not as strongly bound as in the case of the B variant; the rate of catalysis decreases. Therefore, C variant milk is less suitable for the production of sweet-milk cheese than B variant milk.

γ-*Caseins*. These proteins are degradation products of the β-caseins, formed by milk proteases, e. g., γ_1-casein is obtained by cleavage of the residues 1–28. The peptide released is identical to the proteose-peptone PP8F which has been found in milk. Correspondingly, γ_2- and γ_3-caseins are formed by hydrolysis of the amino acid residues 1–105 and 1–107 respectively. According to more recent nomenclature recommendations, β-casein fragments should be described by the position numbers. Thus, γ_1-casein from any β-casein variant X is called, e. g., β-casein X (f29–209) and the corresponding proteose peptone PF8F β-casein X (f1–28).

λ-*Caseins*. The λ-casein fraction consists mainly of fragments of the α_{s1}-caseins. In vitro the α-caseins are formed by incubation of the α_{s1}-caseins with bovine plasmin.
The molar ratio of the main components $\alpha_{s1}/\beta + \gamma/\kappa/\alpha_{s2}$ is on an average 8/8/3/2. All casein forms contain phosphoric acid, which always occurs in a tripeptide sequence pattern (Pse = phosphoserine):

$$\text{Pse-X-Glu} \quad \text{or} \quad \text{Pse-X-Pse} \tag{10.5}$$

in which X is any amino acid, including phosphoserine and glutamic acid. Examples are:

α_{s1}-Casein :	Pse-Glu-Pse
	Pse-Ile-Pse-Pse-Pse-Glu
	Pse-Val-Glu
	Pse-Ala-Glu
β-Casein :	Pse-Leu-Pse-Pse-Pse-Glu
	Pse-Glu-Glu
κ-Casein :	Pse-Pro-Glu

(10.6)

Most probably this regular pattern originates from the action of a specific protein kinase. The various distribution of polar and apolar groups of the individual proteins outlined above are summarized in Table 10.11. The hydrophobicity values listed are average hydrophobicity values $\overline{H}$ of the amino acid side chains present in the sequence of the given segments, and are calculated as follows:
A measure of the hydrophobicity of a compound is the free energy, F_t, needed to transfer the compound from water into an organic solvent, and is given as the ratio of the compound's solubility in water (N_w, as mole fraction) and in the organic solvent (N_{org}, as mole fraction), involving the ac-

Table 10.11. Distribution of amino acid residues with ionizing side chains (net charge) and with nonpolar side chains (hydrophobicity) in α_{s1}-casein and β-casein

Residue	α_{s1}-Casein		Residue	β-Casein	
	1	2		1	2
1–40	+3	1340	1–43	−16	783
41–80	−22.5	641	44–92	−3.5	1429
81–120	0	1310	93–135	+2	1173
121–160	−1	1264	136–177	+3	1467
161–199	−2.5	1164	178–209	+2	1738

1 Net charge.
2 Hydrophobicity $\overline{H}$ (Cal/mole; cf. text).

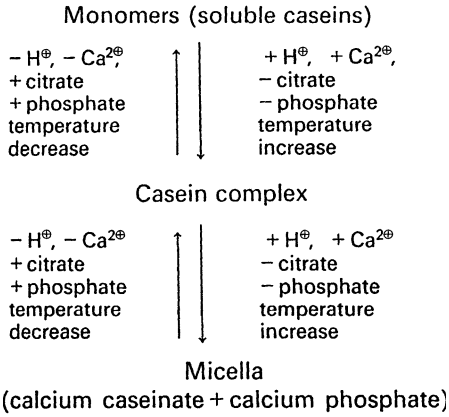


Fig. 10.3. Casein complex and casein micelle formation

tivity coefficients (γ_w, γ_{org}):

$$\Delta F_t = RT \ln \frac{N_w \cdot \gamma_w}{N_{org} \cdot \gamma_{org}} \quad (10.7)$$

The corresponding free energy of transfer of the side chain of an amino acid $H\Phi_i$ is obtained from the following relationship:

$$H\Phi_i = \Delta F_t(\text{amino acid i}) - \Delta F_i(\text{glycine})$$

The average hydrophobicity of a sequence segment of a polypeptide chain with n amino acid residues is then:

$$\overline{H} = \frac{\Sigma H\Phi_i}{n} \quad (10.8)$$

The higher the $H\Phi_i$, i. e. $\overline{H}$, the higher is the hydrophobicity of individual side chains, i. e. the sequence segment. Data provided in Table 10.11 are related to the ethanol/water system.

10.1.2.1.2 Micelle Formation

Only up to 10% of the total casein fraction is present as monomers. They are usually designated as serum caseins and the concentration ratio $c_\beta > c_\kappa > c_{\alpha sl}$ is quite valid. However, the main portion is aggregated to casein complexes and casein micelles. This aggregation is regulated by a set of parameters, as presented in Fig. 10.3. Dialysis of casein complexes against a chelating agent might shift the equilibrium completely to monomers, while against high Ca^{2+} ion concentrations the shift would be to large micelles.

From Fig. 10.4 it follows that the diameter of the micelles in skim milk varies from 50–300 nm, with a particle distribution peak at 150 nm. Using an average diameter of 140 nm, the micelle volume is 1.4×10^6 nm^3 and the particle weight is 10^7–10^9dal. This corresponds to 25,000 monomers per micelle. Casein micelles are substantially smaller than fat globules, which have diameters between 0.1–10 µm. Scanning electron micrographs of micelles are shown in Fig. 10.5 and compositional data are provided in Table 10.12.

The ratio of monomers in micelles varies to a great extent (Table 10.13), depending on dairy cattle breed, season and fodder, and is influenced also by micellular size (Table 10.14). The micelles are not tightly packed and so are of variable density. They are strongly solvated

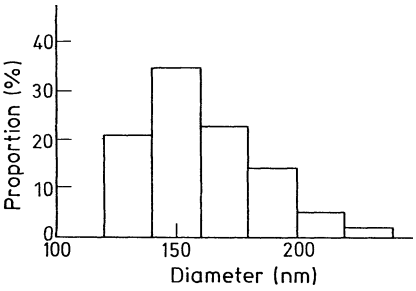


Fig. 10.4. Particle size distribution of casein micelles in skim milk (fixation with glutaraldehyde)

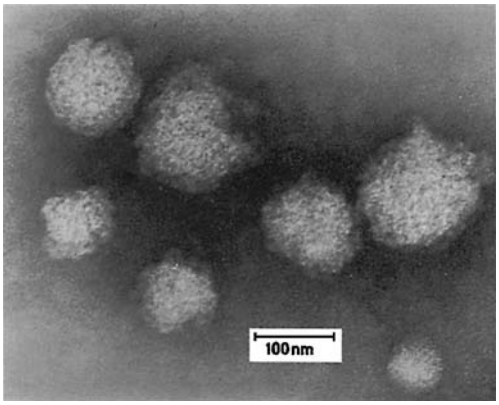


Fig. 10.5. Electron micrograph of the casein micelles in skim milk (according to *Webb*, 1974). The micelles are fixed with glutaraldehyde and then stained with phosphomolybdic acid

Table 10.12. Composition of casein micelles (%)

Casein	93.2	Phosphate	
Ca	2.9	(organic)	2.3
Mg	0.1	Phosphate	
Na	0.1	(inorganic)	2.9
K	0.3	Citrate	0.4

Table 10.13. Typical distribution of components in casein micelles

Component	Ratio numbers			
α_{s1}	3	6	9	12
β	1	1	4	4
γ		1	1	1
κ	1	3	3	3

(1.9 g water/g protein) and hence are porous. The monomers are kept together with:

- Hydrophobic interactions that are minimal at a temperature less than 5 °C.
- Electrostatic interactions, mostly as calcium or calcium phosphate bridges between phosphoserine and glutamic acid residues (Fig. 10.6).
- Hydrogen bonds.

On a molecular level different micelle models have been proposed which to a certain extent explain the experimental findings. The most probable model is shown in Fig. 10.7. This model comprises subunits (submicelles, $M_r \sim 760{,}000$)

Table 10.14. Composition and size of casein micelles isolated by centrifugation

Centrifugation time (min)[a]	Composition of the sediment(%)			
	α_{s1}	β	κ	Others
0[b]	50	32	15	3
0–7.5	47	34	16	3
7.5–15	46	32	18	4
15–30	45	31	20	4
39–60	42	29	26	3
Serum casein	39	23	33	5

[a] Centrifugation speed $10^5 \times$ g.
[b] Isoelectric casein.

which consist of ca. 30 different casein monomers and aggregate to large micelles via calcium phosphate bridges. Two types of subunits apparently exist: one type contains κ-casein and the other does not. The κ-casein molecules are arranged on the surface of the corresponding submicelles. At various positions, their hydrophilic C-termini protrude like hairs from the surface, preventing aggregation. Indeed, aggregation of the submicelles proceeds until the entire surface of the forming micelle is covered with κ-casein, i. e., covered with "hair", and, therefore, exhibits steric repulsion. The effective density of the hair layer is at least 5 nm. A small part of the κ-casein is also found inside the micelle.

10.1.2.1.3 Gel Formation

The micelle system, can be destabilized by the action of rennin or souring. Rennin attacks κ-casein, eliminating not only the C-terminus in the form of the soluble glycopeotide 106–169, but also the cause of repulsion. The remaining paracasein micelles first form small aggregates with an irregular and often long form, which then assemble with gel formation to give a three dimensional network with a pore diameter of a few μm. The fat globules present are included in this network with pore enlargement. It is assumed that dynamic equilibria exist between casein monomers and submicelles, dissolved and bound calcium phosphate, and submicelles and micelles.

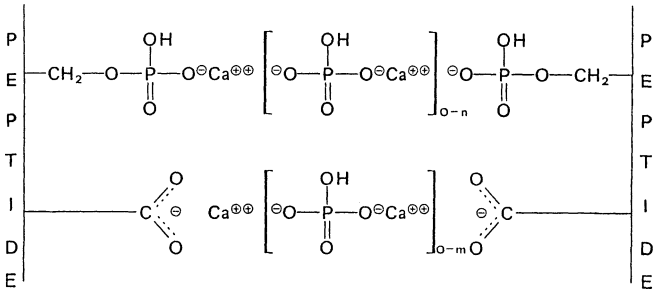


Fig. 10.6. Peptide chain bridging with calcium ions

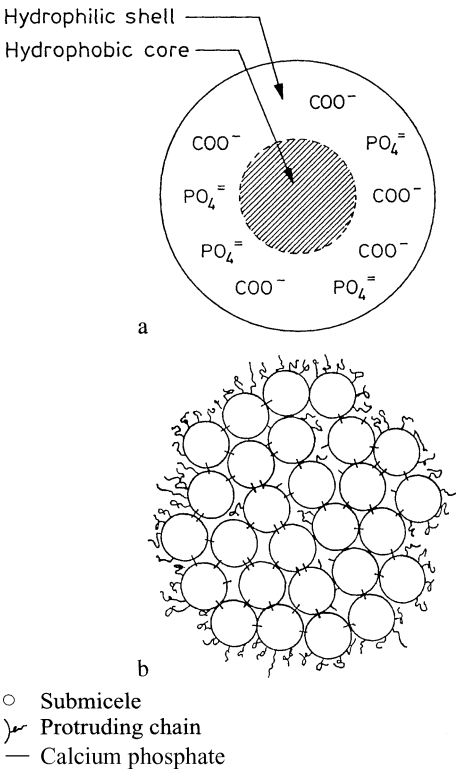


Fig. 10.7. Schematic model of a casein micelle; (**a**) a subunit consisting of α_{s1}-, β-, γ-, κ-caseins, (**b**) Micelle made of subunits bound by calcium phosphate bridges (according to *Webb*, 1974)

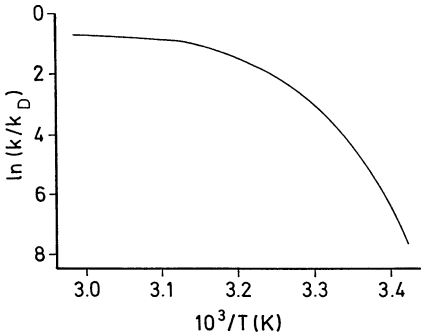


Fig. 10.8. Temperature dependency of the aggregation rate of para-casein micelles (rate constant k in fractions of the diffusion-controlled rate k_D; according to *Dalgleish*, 1983)

The rate of gel formation increases with increasing temperature (Fig. 10.8). It is slow at $T < 25\,^{\circ}C$ and proceeds almost under diffusion control at $T \sim 60\,^{\circ}C$. It follows that hydrophobic interactions, especially due to the very hydrophobic para-κ-casein remaining on the surface after the action of rennin, are the driving force for gel formation. In addition, other temperature-dependent reactions play a role, like the binding of calcium ions and of β-casein to the micelles, and the change in solubility of colloidal calcium phosphate.

Acid coagulation of casein is also definitely caused by hydrophobic interactions, as shown by the dependency of the coagulation rate on the temperature and pH value (Fig. 10.9). On acidification, the micelle structure changes due to the migration of calcium phosphate and monomeric casein. Since the size of the micelle remains practically constant, this migration of components must be associated with swelling. During coagulation, dissolved casein reassociates with the micelles, forming a gel network.

The gel structure can be controlled via changes in the hydrophobicity of the micelle surface. A decrease in hydrophobicity is possible, e. g., by heating milk (90 °C/10 min). Covalent bonding of denatured β-lactoglobulin to κ-casein (cf. 10.1.3.5) occurs, burying hydrophobic groups.

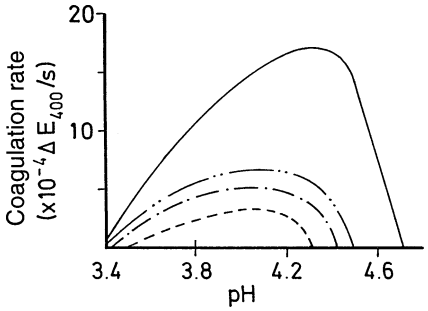


Fig. 10.9. Rate of coagulation of casein micelles as a function of temperature and pH value (—— 25 °C, – ·· – 15 °C, – · – 10 °C, – – – 5 °C, according to *Bringe, Kinsella*, 1986)

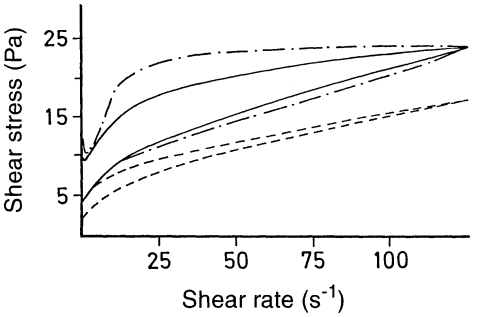


Fig. 10.11. Flow curves of stiff skim-milk yoghurt subjected to defined prestirring as a function of the rate of denaturation of β-lactoglobulin B (temperature/time/denaturation rate 90 °C/2.2 s/10%: – – –, 90 °C/21 s/60%: ——, 90 °C/360 s/99%: – . –; according to *Kessler*, 1988)

Due to weaker interactions, stable, rigid gels with a chain-like structure are formed on acidification. These gels exhibit no syneresis and are desirable, e. g., in yoghurt (10.2.1.2). Figure 10.10 shows that the firmness of stiff yoghurt is highest when the denaturation of β-lactoglobulin is 90–99%. If this rate of denaturation is achieved at lower temperatures (e. g., 85 °C), gels are formed that are more rigid and coarser than those formed by heating to higher temperatures (e. g., 130 °C), which results in a soft, smooth gelatinous mass. The gel stability of whole-milk yoghurt is lower than that of skim-milk yoghurt because the protein network is interrupted by included fat globules.

Flow curves of skim-milk yoghurt as a function of the rate of denaturation of β-lactoglobulin are presented in Fig. 10.11. The yield point is a measure of the elastic properties of the gel and the area enclosed by the hysteresis loop is a measure of the total energy required to destroy the gel. Both parameters increase with increasing rate of denaturation, which is a sign of increasing gel stability. In contrast to yoghurt production, syneresis of the gel is desirable in the production of cottage cheese, so that the typical texture is attained. For this reason, the milk is only slightly heat treated and the surface hydrophobicity is increased by the addition of chymosin before acidification.

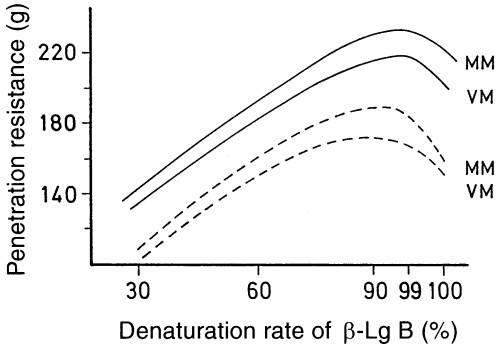


Fig. 10.10. Firmness of yoghurt as a function of the rate of denaturation of β-lactoglobulin B (the final value of the penetration resistance of a conical test piece in stiff yoghurt is given; heating temperature 85 °C: ——, 130 °C: – – –, WM: whole milk with 3.5% fat, SM: skim milk; according to *Kessler*, 1988)

10.1.2.1.4 Whey Proteins

β-*Lactoglobulin* occurs in genetic variants A, B and C of the Jersey dairy cattle breed, and variant D of the Montbeliarde dairy cow. Two other A_{Dr} and B_{Dr} variants of Australian drought master cows are identical to variants A and B apart from the carbohydrate content.

Table 10.9 shows the corresponding changes in the amino acid composition of β-lactoglobulin.

The monomeric β-lactoglobulin has a molecular weight of 18 kdal and consists of 162 amino acids, whose sequence is shown in Table 10.8. It exhibits a reversible, pH-dependent oligomerization, as represented by the equation:

$$A \rightleftarrows A_2 \rightleftarrows (A_2)_4 \rightleftarrows A_2 \rightleftarrows A$$
$$pH < 3.5 \quad 3.7 < pH < 5.1 \quad pH > 7.5 \qquad (10.9)$$

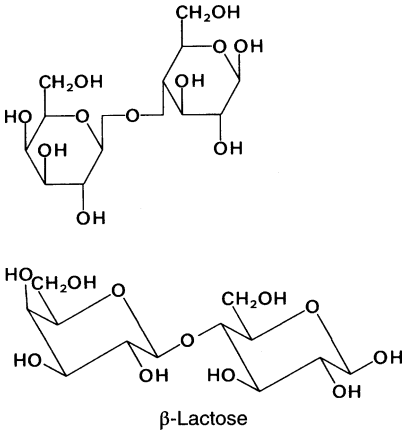
Hence, the monomer is stable only at a pH less than 3.5 or above 7.5. The octamer occurs with variant A, but not with variants B and C. Irreversible denaturation occurs at a pH above 8.6 as well as by heating or at higher levels of Ca^{2+} ions. β-lactoglobulin has 5 cysteine residues, one (Cys^{121}, Table 10.8) of which being free. In the native protein, however, this cysteine is buried within the structure. This SH group is exposed on partial denaturation and can participate either in protein dimerization via disulfide bridge formation or in reactions with other milk proteins, especially with κ-casein and α-lactalbumin, which proceed during the heating of milk.

α-Lactalbumin (M_r 14,200). This protein exists in two genetic forms, A and B (Gln → Arg). It has 8 cysteine residues. Its amino acid sequence (Table 10.8), which is similar to that of lysozyme, has been elucidated. Disulfide bonds and a $Ca^{2\oplus}$ ion participate in the stabilization of the tertiary structure. α-Lactalbumin has a biological function since it is the B subunit of the enzyme lactose synthetase. The enzyme subunit A is a nonspecific UDP-galactosyl transferase; the subunit B makes sure that the transfer of the galactose residue can occur at the low glucose concentration present in mammals. The affinity of the transferase alone for glucose is too low ($K_m = 2$ mol/l). It is increased 1000 fold by cooperation with α-lactalbumin.

10.1.2.2 Carbohydrates

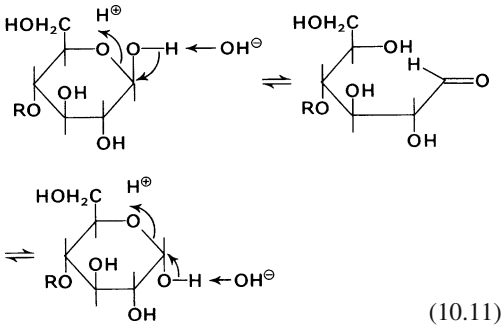
The main sugar in milk is lactose, an O-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose, which is 4–6% of milk.

The most stable form is α-lactose monohydrate, $C_{12}H_{22}O_{11} \cdot H_2O$. Lactose crystallizes in this form from a supersaturated aqueous solution at $T < 93.5\,^{\circ}C$. The crystals may have a prism- or pyramid-like form, depending on conditions. Vacuum drying at $T > 100\,^{\circ}C$ yields a hygroscopic α-anhydride. Crystallization from aqueous solutions above 93.5 °C provides water-free β-lactose (β-anhydride, cf. Formula 10.10). Rapid drying of a lactose solution, as in milk powder production, gives a hygroscopic and amorphous equilibrium mixture of α- and β-lactose.



(10.10)

Some physical data of lactose are summarized in Table 10.15. The ratio of anomers is temperature dependent. As temperature increases, the β-form decreases. The mutarotation rate is temperature ($Q_{10} = 2.8$) and pH dependent (Fig. 10.12). The rise in mutarotation rate at pH < 2 and pH > 7 originates from the rate-determining step of ring opening, which is catalyzed by both H^+ and OH^- ions:



(10.11)

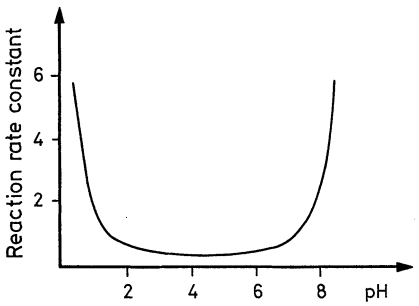


Fig. 10.12. Mutarotation rate of lactose as affected by pH

Table 10.15. Some physical characteristics of lactose

	α-Lactose	β-Lactose	Equilibrium mixture
Melting point (°C)	223[a]	252.2[a]	
Spec. rotation $[\alpha]_D^{20}$	89.4	35.0	
Equilibrium in aqueous solution[b]			
0 °C	1.00	1.80	
20 °C	1.00	1.68	
50 °C	1.00	1.63	
Solubility in water[c]			
0 °C	5.0	45.1	11.9
25 °C	8.6		21.6
39 °C	12.6		31.5
100 °C	70	94.7	157.6

[a] Anhydrous. [b] Relative concentration.
[c] g Lactose/100 g water.

The great solubility difference between the two anomers is noteworthy. The sweetness of lactose is significantly lower than that of fructose, glucose or sucrose (Table 10.16). For people who suffer under lactose intolerance, dietetic milk products are produced by treatment with β-1,4-galactosidase (cf. 2.7.2.2.7). Glucose and some other amino sugars and oligosaccharides are present in small amounts in milk.

Lactulose is found in heated milk products. It is a little sweeter and clearly more soluble than lactose. For example, condensed milk contains up to 1% of lactulose, corresponding to an isomerization of ca. 10% of the lactose present. The formation proceeds via the *Lobry de Bruyn–van Ekenstein* rearrangement (cf. 4.2.4.3.2) or via *Schiff* base. Traces of epilactose (4-O-β-D-glactopyranosyl-D-mannose) are also formed on heating milk.

Table 10.16. Relative sweetness of saccharose, glucose, fructose and lactose[a]

Saccharose	Glucose	Fructose	Lactose
0.5	0.9	0.4	1.9
5.0	8.3	4.2	15.7
10.0	12.7	8.7	20.7
20.0	21.8	16.7	33.3

[a] Results are expressed as concentration % for isosweet aqueous sugar solutions.

10.1.2.3 Lipids

The composition of milk fat is presented in Table 10.17. Milk fat contains 95–96% triglycerols. Its fatty acid composition is given in Table 10.18. The relatively high content of low molecular weight fatty acids, primarily of butyric acid, is characteristic of milk. Although linoleic acid dominates in the lipids occurring in feed, the content of this fatty acid is very low in milk fat (Table 10.17). The reason was found to be that microorganisms living in the rumen hydrogenate the linoleic acid to oleic acid and stearic acid with the formation of conjugated linoleic acid (CLA, cf. 3.2.1.2) and vaccenic acid as intermediates, as shown in Fig. 10.13. It is possible to increase the concentration of linoleic

Table 10.17. Milk lipids

Lipid fraction	Percent of the total lipid
Triacylglycerols	95–96
Diacylglycerols	1.3–1.6
Monoacylglycerols	0.02–0.04
Keto acid glycerides	0.9–1.3
Hydroxy acid glycerides	0.6–0.8
Free fatty acids	0.1–0.4
Phospholipids	0.8–1.0
Sphingolipids	0.06
Sterols	0.2–0.4

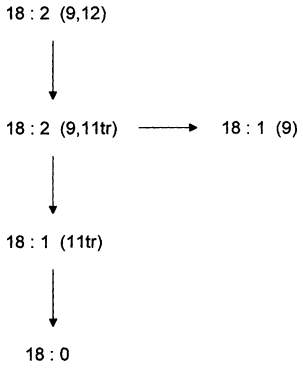


Fig. 10.13. Biohydrogenation of linoleic acid in the rumen of ruminants

Table 10.18. Fatty acid composition of milk fat[a]

Fatty acid	Weight-%
Saturated, straight chain	
Butyric acid	2.79
Caproic acid	2.34
Caprylic acid	1.06
Capric acid	3.04
Lauric acid	2.87
Myristic acid	8.94
Pentadecanoic acid	0.79
Palmitic acid	23.8
Heptadecanoic acid	0.70
Stearic acid	13.2
Nonadecanoic acid	0.27
Arachidic acid	0.28
Behenic acid	0.11
Saturated, branched chain	
12-Methyltetradecanoic acid	0.23
13-Methyltetradecanoic acid	0.14
14-Methylpentadecanoic acid	0.20
14-Methylhexadecanoic acid	0.23
15-Methylhexadecanoic acid	0.36
3,7,11,15-Tetramethylhexadecanoic acid	0.12–0.18
Unsaturated	
9-Decenoic acid	0.27
9-cis-Tetradecenoic acid	0.72
9-cis-Hexadecenoic acid	1.46
9-cis-Heptadecenoic acid	0.19
8-cis-Octadecenoic acid	0.45
Oleic acid	25.5
11-cis-Octadecenoic acid	0.67
9-trans-Octadecenoic acid	0.31
10-trans-Octadecenoic acid	0.32
11-trans-Oxtadecenoic acid	1.08
12-trans-Octadecenoic acid	0.12
13-trans-Octadecenoic acid	0.32
14-trans-Octadecenoic acid	0.27
15-trans-Octadecenoic acid	0.21
16-trans-Octadecenoic acid	0.23
Linoleic acid	2.11
Linolenic acid	0.38

[a] Only acids with a content higher than 0.1% are listed.

acid in milk fat, e. g., by adding plant fats of the appropriate composition in encapsulated form to the feed. The disadvantage of such a nutritionally/physiologically interesting approach is the changed physico-chemical properties of the dairy product, e. g., an increased susceptibility to oxidation and the formation of unsaturated lactones (γ-dodec-cis-6-enolactone from linoleic acid) which influences the flavor of milk and meat. In addition to the main straight-chain fatty acids, small amounts of odd-C-number, branched-chain and oxo-fatty acids (cf. 3.2.1.3) are present.

Phospholipids are 0.8–1.0% of milk fat and sterols, mostly cholesterol, are 0.2–0.4%. Butterfat melting properties, as affected by season and fodder, are listed in Table 10.19. Milk fat is very finely distributed in plasma. The diameter of fat globules is 0.1–10 μm, but for the main part in the range of 1–5 μm. During homogenization, milk at 50–75 °C is forced through small passages under pressure of up to 35 MPa, the diameter of the globules lowers to <1 μm, depending on the pressure. The fat droplets are surrounded by a membrane that consists of phospholipids and a double layer of proteins and accounts for about 2% of the total mass of the droplet. The layer thickness is on average 8–9 nm, but is not uniform. Membrane compositional data are given in Table 10.20.

About 40 proteins with M_r 15–240 kdal (milk fat globule membrane proteins, MFGM proteins) are involved in the make-up of the membrane. Although their nutritional value with regard to the caseins and whey proteins is low, they receive

Table 10.19. Melting characteristics of butterfat

Temperature (°C)	Solid content (%)	Temperature (°C)	Solid content (%)
5	43–47	30	6–8
10	40–43	35	1–2
20	21–22	40	0

Table 10.20. Membrane composition of milkfat globules

Constituent	Proportion (%)
Protein	41
Phospho- and glycolipids	30
Cholesterol	2
Neutral glycerides	14
Water	13

attention because they can be detrimental to the health of sensitized persons. Casein proteins enter and participate in membrane formation when the fat globule surface area is expanded 4- to 6-fold during homogenization of milk. Six of the eight dominant proteins in the MFGM are glycoproteins, e. g., xanthine oxidoreductase. Other enzymes which are present in the membrane are acetylcholine esterase as well as alkaline and acidic phosphatase (cf. Table 10.24). A very active lipoprotein lipase, a glycoprotein (8.3% carbohydrate, molecular weight 48.3 kdal), occurs in the casein micelles. However, if the milking and storage procedures are appropriate, the raw milk can be kept for several days without the development of a rancid off-flavor. It is likely that the membranes of the fat globules prevent lipolysis. Disruption of the organized structure of the membrane, for instance by homogenization, allows the lipase to bind to the fat globules and to hydrolyze the triacylglycerols at a high rate (1 µmole fatty acid per min per ml milk, pH 7, 37 °C). The milk becomes unpalatable within a few minutes. Therefore the lipoprotein lipase has to be inactivated by pasteurization prior to milk homogenization.

The small amounts of gangliosides that occur in milk (5.6 µmol/l, calculated as ganglioside-bound sialic acid) are of interest for the analytical differentiation of skim-milk and butter-milk powder. As structural elements of the membrane of the fat globules, the cream gets enriched with gangliosides during skimming and only about 8% remain in the skimmed milk. During butter-making, the membrane of the fat globules is mechanically destroyed and the highly polar gangliosides pass almost completely into the buttermilk. Therefore, unlike skim-milk powder, butter-milk powder is rich in gangliosides (ca. 480 µmol/kg, calculated as sialic acid).

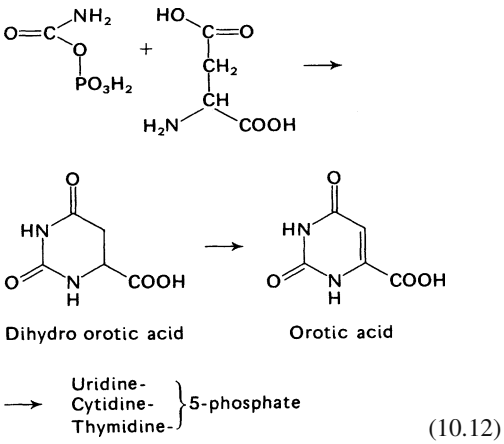
10.1.2.4 Organic Acids

Citric acid (1.8 g/l) is the predominant organic acid in milk. During storage it disappears rapidly as a result of the action of bacteria. Other acids (lactic, acetic) are degradation products of lactose. The occurrence of orotic acid (73 mg/l), an intermediary product in biosynthesis of pyrimidine nucleotides, is specific for milk:

Table 10.21. Indicators for the proportion of milk in foods

Compound	Whole milk powder	Skim milk powder
Orotic acid		
photometric	50.6	66.4
polarographic	46.6	58.1
Total creatinine	66.3	84.4
Uric acid	12.4	15.3

Expressed as mg/100 g solids.



(10.12)

Orotic acid as well as total creatinine and uric acid are suitable indicators for the determination of the proportion of milk in foods. The average values for whole-milk and skim-milk powder given in Table 10.21 can serve as reference values.

10.1.2.5 Minerals

Minerals, including trace elements, in milk are compiled in Table 10.22.

10.1.2.6 Vitamins

Milk contains all the vitamins in variable amounts (Table 10.23). During processing, the fat-soluble vitamins are retained by the cream, while the water-soluble vitamins remain in skim milk or whey.

Table 10.22. Mineral composition of milk

Constituent	mg/l	Constituent	µg/l
Potassium	1500	Zinc	4000
Calcium	1200	Aluminum	500
Sodium	500	Iron	400
Magnesium	120	Copper	120
Phosphate	3000	Molybdenum	60
Chloride	1000	Manganese	30
Sulfate	100	Nickel	25
		Silicon	1500
		Bromine	1000
		Boron	200
		Fluorine	150
		Iodine	60

Table 10.23. Vitamin content of milk

Vitamin	mg/l	Vitamin	mg/l
A (Retinol)	0.4	Nicotinamide	1
D (Calciferol)	0.001	Pantothenic acid	3.5
E (Tocopherol)	1.0	C (Ascorbic acid)	20
B_1 (Thiamine)	0.4	Biotin	0.03
B_2 (Riboflavin)	1.7	Folic acid	0.05
B_6 (Pyridoxine)	0.6		
B_{12} (Cyano-cobalamine)	0.005		

10.1.2.7 Enzymes

Milk contains a great number of enzymes which are not only of analytical importance for the detecton of heat-treated milk, but can also influence the processing properties. The rate of heat inactivation of the enzymes indicates the type and extent of heating (cf. 2.5.4).
Hydrolases identified include: amylases, lipases, esterases, proteinases and phosphatases. Proteinase inhibitors have also been found. Important oxidoreductases in milk are aldehyde dehydrogenase (xanthine oxidase), lactoperoxidase and catalase. A general idea of the occurrence and localization of enzymes in boving milk is given in Table 10.24

10.1.2.7.1 Plasmin

The serine endopeptidase plasmin is of special interest in milk technology.

Table 10.24. Enzymes in bovine milk

EC Number	Name	Localization[a]
1.1.1.27	L-Lactate dehydrogenase	P
1.1.1.37	Malate dehydrogenase	
1.1.3.22	Xanthine oxidase	F
1.4.3.6	Amine oxidase (copper-containing)	
1.6.99.3	NADH dehydrogenase	F
1.8	Sulfhydryl oxidase[b]	S
1.8.1.4	Dihydrolipoamide dehydrogenase	F
1.11.1.6	Catalase	L
1.11.1.7	Lactoperoxidase	S
1.15.1.1	Superoxide dismutase	
2.3.2.2	γ-Glutamyltransferase	F
2.4.1.22	Lactose synthase	S
2.4.99.1	β-Galactoside-α-2,6-sialytransfersase	
2.6.1.1	Aspartate aminotransferase	P
2.6.1.2	Alanine aminotransferase	
2.7.1.26	Riboflavin kinase	
2.7.1.30	Glycerol kinase	
2.7.7.2	FMN adenyl transferase	
2.8.1.1	Thiosulfate sulfurtransferase	
3.1.1.1	Carboxylesterase	S
3.1.1.2	Arylesterase	
3.1.1.7	Acetylcholine esterase	F
3.1.1.8	Choline esterase	S
3.1.1.34	Lipoproteinlipase	C
3.1.3.1	Alkaline phosphatase	F
3.1.3.2	Acid phosphatase	F
3.1.3.5	5′-Nucleotidase	F
3.1.3.9	Glucose-6-phosphatase	F
3.1.3.16	Phosphoprotein phosphatase	P
3.1.4.1	Phosphodiesterase I	F
3.1.27.5	Pancreatic ribonuclease	S
3.2.1.1	α-Amylase	S
3.2.1.2	β-Amylase	
3.2.1.17	Lysozyme	S
3.2.1.24	α-Mannosidase	
3.2.1.31	β-Glucuronidase	
3.2.1.52	β-N-Acetylglucosaminidase	
3.4	Acid peptidases	
3.4.21.7	Plasmin	C
3.6.1.3	Adenosinetriphosphatase	F
3.6.1.9	Nucleotide pyrophosphatase	
4.1.2.13	Fructosebiphosphate aldolase	
5.3.1.9	Glucose-6-phosphate isomerase	

[a] C: casein micelle, F: fat-globule membrane, L: leucocytes, P: plasma, S: serum.
[b] Not thiol oxidase EC 1.8.3.2.

Plasmin, its precursor plasminogen and the plasminogen activator (PA) are present in milk associated to the casein micelles and the membranes of the fat globules. A shift of the pH value to the acidic range (pH 4.7) promotes the release of plasmin from casein. The concentration of plasmin is 0.3–2.5 μg/ml milk. It increases with the age of the cow and during the lactation period.
Plasmin is in the pH range 7.5–8.0 most active at 37 °C. It preferentially hydrolyzes β-casein and at a lower rate also α-casein. κ-Casein is resistant just like the whey proteins α-lactalbumin and β-lactoglobulin. Like trypsin, it attacks the carboxyl side of L-lysine as well as L-arginine on hydrolysis. The activity of plasmin is controlled by the PA inhibitor and the plasmin inhibitor.
The plasmin activity is reduced by only 10–17% under the conditions of pasteurization, e. g., 72 °C for 15 s. Storage of pasteurized milk indirectly promotes plasmin activity because the inhibitors of PA are inactivated. Complete thermal inactivation of plasmin is achieved at 120 °C in 15 min and at 142 °C in 18 s.
Plasmin influences the ripening process, e.g., in Camembert. It is accelerated and aroma formation is improved. In the recovery of caseinates, on the other hand, the separation of plasmin is absolutely necessary.

10.1.2.7.2 Lactoperoxidase

In the preservation of raw milk, the antimicrobial lactoperoxidase (LP) system present in milk is of interest. This LP system offers an alternative, especially in countries where it is not possible to cool the milk to protect it from spoilage.
The system consists of LP (EC 1.11.1.7) and the substrates thiocyanate and H_2O_2. The enzyme, a glycoprotein (carbohydrate content 10%), consists of 612 amino acid residues (M_r 78,000, IP 9.6) and Fe-protoporphyrin IX, which as the prosthetic group carries out the catalysis, as described in 2.3.2.2. Thiocyanate takes part in this reaction process as the electron donor AH. Lactoperoxidase is one of the heat stable enzymes of milk, especially when the structure is fixed by $Ca^{2\oplus}$ ions. It is still active after 30 min at 63 °C (neutral pH) and after 15 s at 72 °C, but it is inactive after only 2.5 s at 80 °C. Acidification (pH 5.3) accelerates the inactivation by liberating the $Ca^{2\oplus}$ ions. After xanthine oxidase, LP is the most common enzyme in milk: ca. 30 mg/l.
The thiocyanate concentration in milk depends on the feed, e. g., on the occurrence of glucosinolates (cf. 17.1.2.9.3). H_2O_2 is not a component of milk, but is supplied by bacteria, e. g., lactic acid bacteria.
Hypothiocyanite is the main product formed from the hydrogen donor thiocyanate in LP catalysis.

$$SCN^{\ominus} + H_2O_2 \xrightarrow{LP} OSCN^{\ominus} + H_2O$$

This compound is a bactericidal agent because it can oxidize the SH groups of structure-forming proteins and enzymes in bacteria. The LP system is used to prolong the shelf life of raw milk and pasteurized milk. H_2O_2 is produced here by the addition of glucose/glucose oxidase (cf. 2.7.2.1.1) and the concentration of thiocyanate is increased by addition.
In the production of fermented milk products, the LP system can inhibit the development of starter cultures.

10.1.3 Processing of Milk

Only a small amount of milk is sold to the consumer without processing (certified raw milk). The main part is subjected to a processing procedure shown schematically in Fig. 10.14.

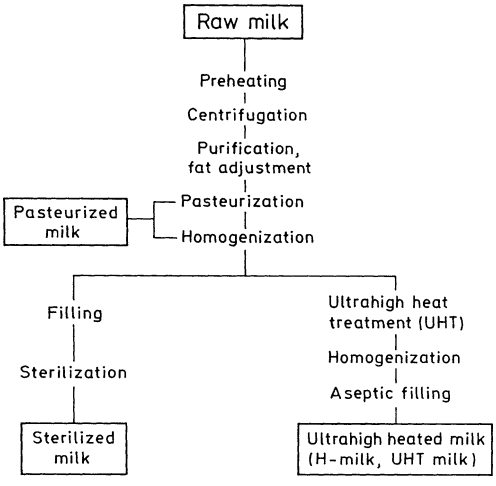


Fig. 10.14. Treatment of milk

10.1.3.1 Purification

The milk is usually delivered in the cooled tank (at least −8 °C of a milk truck. For purification, it is fed into a clarifier (self-cleaning disk separator) via a deaerating vessel. These separators can process either cold or warm milk (40 °C) at speeds of 4500–8400 rpm with throughput capacities of up to 50,000 l/h.

10.1.3.2 Creaming

After heating to about 40 °C (increase in creaming efficiency by lowering the viscosity), the milk is separated into cream and skimmed milk in a cream separator. Cream separators have a nominal capacity of up to 25,000 l/h at speeds of 4700–6500 rpm. The fat content of the milk can be standardized by careful back-mixing.

10.1.3.3 Heat Treatment

The fluid milk is heated to improve its durability and to kill disease-causing microorganisms. Heat treatments used are (cf. Fig. 10.15):

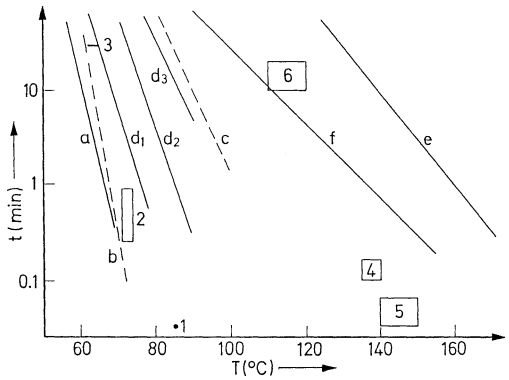


Fig. 10.15. Heating of milk. *1–3* Pasteurization: *1* high temperature treatment, *2* short time and *3* long time heat treatment; *4* and *5* UHT treatment: *4* indirect and *5* direct; *6* sterilization. *a*: Killing pathogenic microorganisms (*Tubercle bacilli* as labelling organism), *b*/*c*: inactivation of alkaline/acid phosphatase. d_1, d_2, d_3 denaturation (5, 40, 100%) of whey proteins. *e*: casein heat coagulation, *f*: start of milk browning

- Thermization.
  The process involves heating under conditions that are milder than those of pasteurization, e. g., 57–68 °C. The number of bacteria is reduced, e. g., for the production of cheese. The taste of the milk and the coagulation time during treatment with rennet are not impaired.
- Pasteurization.
  The milk is treated: at high temperature (85 °C for 2–3 s) in a short-time, flash process (72–75 °C/15–30 s) in plate heaters; or by the low temperature or holder process, in which it is heated at 63–66 °C for at least 30–32 min, with stirring, and is then cooled.
- Ultrahigh Temperature (UHT) Treatment.
  The process involves indirect heating by coils or plates at 136–138 °C for 5–8 s, or direct heating by live steam injection at 140–145 °C for 2–4 s, followed by aseptic packaging.
  To prevent dilution or concentration of the milk, the amount of injected steam must be controlled in such a way that it corresponds to the amount of water withdrawn during expansion under vacuum.
- Bactotherm Process.
  This is a combination of centrifugal sterilization in bactofuges (65 to 70 °C) and UHT heating of the separated sediment (2–3% of the milk), followed by recombination. Since the total amount of milk is not heated in this process, the taste is improved. The storability is ca. 8–10 days.
- Sterilization.
  Milk in retail packages is heated in autoclaves at 107–115 °C/20–40 min, 120–130 °C/8–12 min.

10.1.3.4 Homogenization

Homogenization is conducted to stabilize the emulsion milk by reducing the size of the fat globules. This is achieved by high-pressure homogenization (up to 35 MPa, 50–75 °C). In principle, the high-pressure homogenizer is a high-pressure pump which presses the product through a homogenizing valve. The fat globules are reduced in size to a diameter of <1 µm by turbulence, cavitation and shear forces, resulting in a ca. 10 fold increase in the surface area. The membranes of the reduced fat globules

are formed by the uptake of caseins and whey proteins.

10.1.3.5 Reactions During Heating

Heat treatment affects several milk constituents. Casein, strictly speaking, is not a heat-coagulable protein; it coagulates only at very high temperatures (cf. Fig. 10.15). Heating at 120 °C for 5 h dephosphorylates sodium or calcium caseinate solutions (100% and 85%, respectively) and releases 15% of the nitrogen in the form of low molecular weight fragments.

However, temperature and pH strongly affect casein association and cause changes in micellular structure (cf. 10.1.2.1.2 and 10.1.2.1.3). An example of such a change is the pH-dependent heat coagulation of skim milk. The coagulation temperature drops with decreasing pH (Fig. 10.16 and 10.9). Salt concentration also has an influence, e. g., the heat stability of milk decreases with a rise in the content of free calcium.

All pasteurization processes supposedly kill the pathogenic microorganisms in milk. The inactivation of the alkaline phosphatase is used in determining the effectiveness of pasteurization. At higher temperatures or with longer heating times, the whey proteins start to denature – this coincides with the complete inactivation of acid phosphatase. Denatured whey proteins, within the pH-range of their isoelectric points, cease to the soluble and coagulate together with casein due to souring or chymosin action of the milk. Such coprecipitation of the milk proteins is of importance in some milk processing (as in cottage cheese production). The thermal stability of whey proteins is illustrated in Fig. 10.17.

Heat treatment of milk activates thiol groups; e. g., a thiol-disulfide exchange reaction occurs between κ-casein and β-lactoglobulin. This reaction reduces the vulnerability of κ-casein to chymosin, resulting in a more or less strong retardation of the rennet coagulation of heated milk.

Further changes induced by heating of milk are:

- Calcium phosphate precipitation on casein micelles.
- *Maillard* reactions between lactose and amino groups (e. g. lysine) which, in a classical sterilization process, causes browning of milk and formation of hydroxymethyl furfural (HMF).
- δ-Lactone and methyl ketone formation from glycerides esterified with hydroxy- or keto-fatty acids.
- Degradation of vitamin B_1, B_6, B_{12}, folic acid and vitamin C. Losses of 10–30% in the production of UHT milk are possible. Sterilization destroys ca. 50% of the vitamins B_1, B_6 and folic acid and up to 100% of vitamin C and B_{12}.

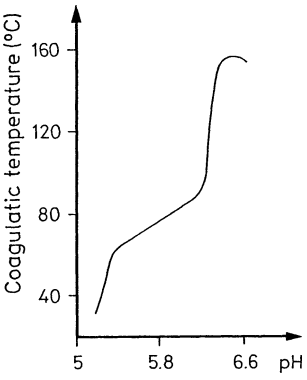


Fig. 10.16. Thermal coagulation of skim milk

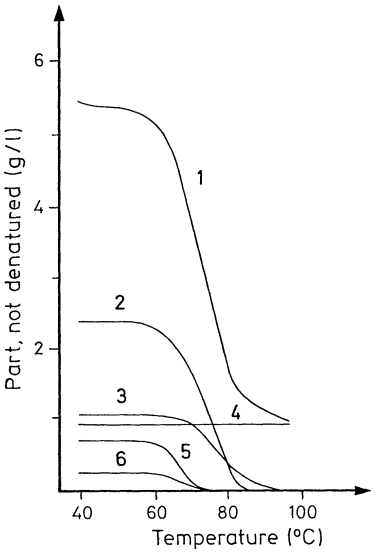


Fig. 10.17. Denaturation of whey proteins by heating at various temperatures for 30 min. *1* Total whey protein, *2* β-lactoglobulin, *3* α-lactalbumin, *4* proteose peptone, *5* immunoglobulin, *6* serum albumin

- Changes in membranes of milk fat globules, which affect the cream separation property of the globules.

Detailed studies have shown that the rate of several reactions which occur during heating of milk, e. g., thiamine and lysine degradations, formation of HMF and nonenzymic browning, can be calculated over a great temperature-time range (including extended storage) by application of a second-order rate law. Assuming an average activation energy of $E_a = 102$ kJ/mole, a "chemical effect" $C^* = 1$ has been defined which gives a straight line in a log t *vs.* T^{-1} diagram, from which the thiamine loss is seen to be approx. 0.8 mg/l (Fig. 10.18). Other lines in Fig. 10.18 represent a power of ten of heat treatment and chemical reactions ($C^* = 10^{-1}, 10^{-2}, \ldots$, or $10^1, 10^2, \ldots$). The pigments formed in a browning reaction become visible only in the range of $C^* = 10$.

Quality deterioration in the form of nutritional degradation, changes in color or development of off-flavor have also been predicted for other foods by application of a suitable mathematical model.

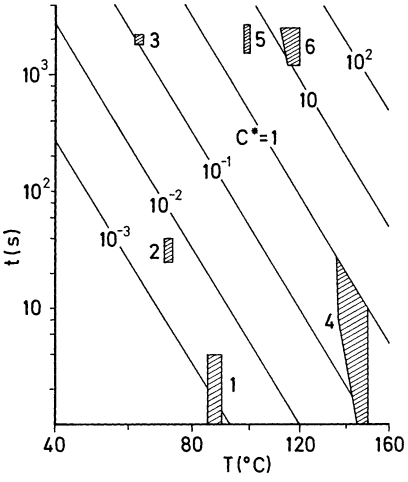


Fig. 10.18. Chemical reactions in heat-treated milk. ("Chemical effect" $C^* = 1$: losses of approx. 3% thiamine and approx. 0.7% lysine and formation of approx. 0.8 mg/l HMF); commonly used heat treatments: *1* high heat, *2* short time heating, *3* prolonged heating, *4* UHT treatment, *5* boiling, *6* sterilization (according to *Kessler*, 1983).
HMF: Hydroxymethylfurfural

In most cases the loss of quality fits a zero- or first-order rate law. Knowledge of the rate constant allows one to predict the extent of reaction for any time.

The influence of temperature on the reaction rate follows the *Arrhenius* equation (cf. 2.5.4). Thus by studying a reaction and measuring the rate constants at two or three high temperatures, one could then extrapolate with a straight line to a lower temperature and predict the rate of the reaction at the desired lower temperature. However, these data allow only a prediction of the shelf life when the physical and chemical properties of the components of a food do not alter with temperature. For example, as temperature rises a solid fat goes into a liquid state. The reactants may be mobile in the liquid fat and not in the solid phase. Thus, shelf life will be underestimated for the lower temperature.

10.1.4 Types of Milk

Milk is consumed in the following forms:

- *Raw fluid milk* (high quality milk), which has to comply with strict hygienic demands.
- *Whole milk* is heat-treated and contains at least 3% fat. It can be a standardized whole milk adjusted to a predetermined fat content, in which case the fat content has to be at least 3.5%.
- *Low-fat milk* is heat-treated and the cream is separated. The fat content is 1.5–2%.
- *Skim milk* is heat-treated and the fat content is less than 0.3%.
- *Reconstituted milk* is most common in regions where milk production is not feasible (e. g. many Japanese cities). For production, melted butter fat is emulsified in a suspension of skim milk powder at 45 °C. The "cream" with a fat content of 20–30% is subjected to two-stage homogenization (20 and 5 MPa, 55–60 °C) and then diluted with the skim milk suspension.
- *Filled milk* is less expensive because the butter fat is replaced with a plant fat.
- *Toned milk* is a blend of a fat-rich fresh milk and reconstitued skim milk in which the nonfat solids are "toned up". Addition of water "tones down" the fat and nonfat solids.

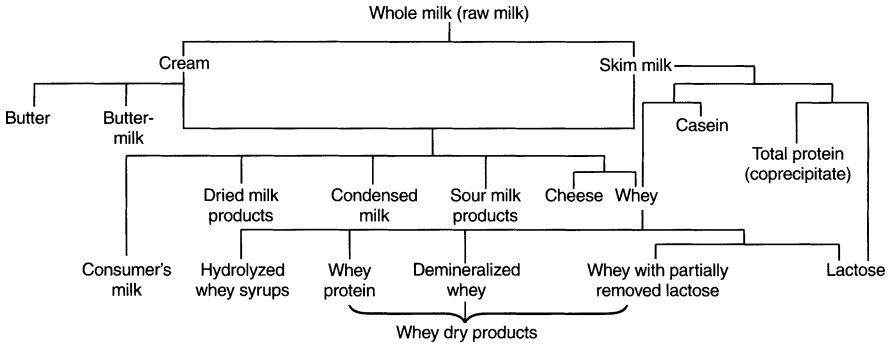


Fig. 10.19. Schematic presentation of milk processing

10.2 Dairy Products

Milk processing is illustrated schematically in Fig. 10.19.

10.2.1 Fermented Milk Products

All sour milk products have undergone fermentation, which can involve not only lacticacid bacteria, but also other microorganisms, e. g., yeasts. To the lactic acid bacteria count the genera *Lactobacillus, Lactococcus, Leuconostoc*, and *Pediococcus.* The most important species are presented in Table 10.25.

Depending on the microorganisms involved, fermentation proceeds via the glycolysis pathway with the almost exclusive formation of lactic acid (homofermentation), via the pentose phosphate pathway with formation of lactic acid, acetic acid (ethanol), and possibly CO_2 (heterofermentation) or via both pathways. These metabolic pathways are shown in Fig. 10.20. Organisms that are obligatorily homofermentative have fructose-bisphosphate aldolase, but not glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. However, organisms that are obligatorily heterofermentative have both dehydrogenases, but no aldolase. Facultatively homofermentative organisms have all three enzymes and can use both metabolic pathways.

Apart from the type of fermentation, the configuration of the lactic acid formed also depends on

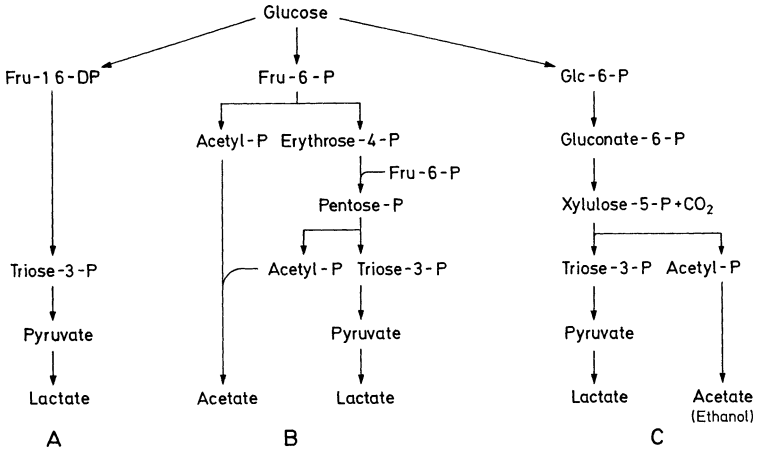


Fig. 10.20. Glucose metabolism in lactic acid bacteria. A: homofermentation, B: Bifidus pathway, and C: heterofermentation (6-phosphogluconate pathway)

Table 10.25. Lactic acid bacteria

Microorganisms	L-Lactic acid[a] (%)	Remarks
Lactobacillus bulgaricus	0.6–4	thermophilic,
L. lactis	0	homofermentative,
L. leichmanii		D-, L- or
L. delbrueckii		D,L-Lactic acid
L. helveticus	70	
L. jugurti		
L. acidophilus	60	
L. casei subsp. casei		mesophilic,
L. casei subsp. alactosus		homofermentative,
L. casei subsp. pseudo plantarum		D-, L- or D,L-Lactic acid
L. casei subsp. rhamnosus		
L. casei subsp. fusiformis		
L. casei subsp. tolerans		
L. plantarum		
L. curvatus		
L. fermentum		heterofermentative,
L. cellobiosus		D,L-Lactic acid
L. brevii		
L. hilgardii		
L. vermiformis		
L. reuteri		
Streptococcus thermophilus	99	thermophilic, homofermentative
S. faecium		homofermentative
Lactococcus lactis subsp. lactis	92–99	mesophilic, homofermentativ
Lactococcus lactis subsp. cremoris	99	
Leuconostoc cremoris		heterofermentative,
L. mesenteroides		D-Lactic acid
L. dextranicum		
L. lactis		
Pediococcus acidilactici		thermophilic, homofermentative, D,L-Lactic acid

[a] Orientation values; the proportion of L-lactic acid depends on the bacterial strain and on the culture conditions.

the microorganisms involved. As shown in Table 10.25, both enantiomers are formed in varying amounts. Table 10.26 lists the content of total lactic acid and L-lactic acid in various dairy products.

In human metabolism, L-lactic acid is formed exclusively and only one L-lactate dehydrogenase is available. Therefore, the intake of larger amounts of D-lactic acid can result in enrichment in the blood and hyperacidity of the urine. For this reason, the WHO recommends a limitation of the intake of D-lactic acid to 100 mg per day and kg of body weight. Apart from the main products mentioned, various aroma substances are formed in the course of fermentation (cf. 10.3.3). In addition, proteolytic and lipolytic processes occur to a certain extent. During proteolysis, peptides can be formed which have opiate activity and hypotensive, immune-stimulating or antimicrobial effects (cf. Literature).

According to the consistency, a distinction is made between stiff, gel-like products, stirred, creamy products, and drinkable, flowable products. The thermal pretreatment of milk influences the rheological properties of the products as described in section 10.1.2.1.3. The keeping time of sour milk products can be increased if they are produced and filled under aseptic conditions or produced under normal conditions but subsequently heat treated.

Table 10.26. Total lactic acid and L-lactic acid in some dairy products

Product	Total lactic acid (%)	L-Lactic acid (%)[b]
Sour milk	0.97	88
Buttermilk	0.86	87
Sour cream	0.86	96
Joghurt	1.08	54
Curd	0.59	94
Cottage cheese	0.34	92
Emmental	0.27	76
Sbrinz	1.53	58
Tilset cheese	1.27	52

[a] Average values. [b] Based on total lactic acid.

10.2.1.1 Sour Milk

Sour milk is the product obtained by the fermentation of milk, which occurs either by spontaneous souring caused by various lactic-acid-producing bacteria or on addition of mesophilic microorganisms (*Lactococcus lactis, L. cremoris, L. diacetylactis, Leuconostoc cremoris*) to heated milk at 20 °C. As fermentation proceeds, lactose is transformed into lactic acid, which coagulates casein at pH 4–5. The thick, sour-tasting curdled milk is manufactured from whole milk (at least 3.5% milk fat), low-fat milk (1.5–1.8% fat) or from skim milk (at most 0.3% fat), often by blending with skim milk powder to increase the total solids content and to improve the resultant protein gel structure. Sour milk contains 0.5–0.9% of lactic acid. In some countries sheep, water buffalo, reindeer or mare's milk are also processed. Sour cream is produced by a process very similar to that used in sour milk manufacture except that coffee grade cream is used as the raw material.

10.2.1.2 Yoghurt

The production of yoghurt is presented schematically in Fig. 10.21. Yoghurt cultures consist of thermophilic lactic acid bacteria that live together symbiotically (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). Incubation is conducted on addition of 1.5–3% of the operating culture at 42–45 °C for about 3 h. The final product has a pH value of about 4–4.2 and contains 0.7–1.1% of lactic acid. Functional foods include yoghurts which have been incubated with probiotics. Probiotics are defined, cultured strains of lactic acid bacteria, which have been isolated from human intestinal flora, e. g., certain lactobacilli and bifidobacteria. On consumption, they are supposed to reach the large intestine and contribute to the formation of an optimal intestinal flora.

The variety of products is increased by the addition of fruits and fruit pastes to yoghurt.

The addition of fruit or fruit pastes and sugar yields special products (fruit yoghurts).

An essential part of the specific yoghurt aroma comes from carbonyl compounds, predominantly acetaldehyde and diacetyl. In addition to 1-octen-3-one, 1-nonen-3-one has also been detected as an important odorant, which has an exceptionally low odor threshold (cf. 3.7.2.1.9). An autoxidation product of linoleic acid, (E)-2-nonenal (Formula 10.13), is thought to be the precursor.

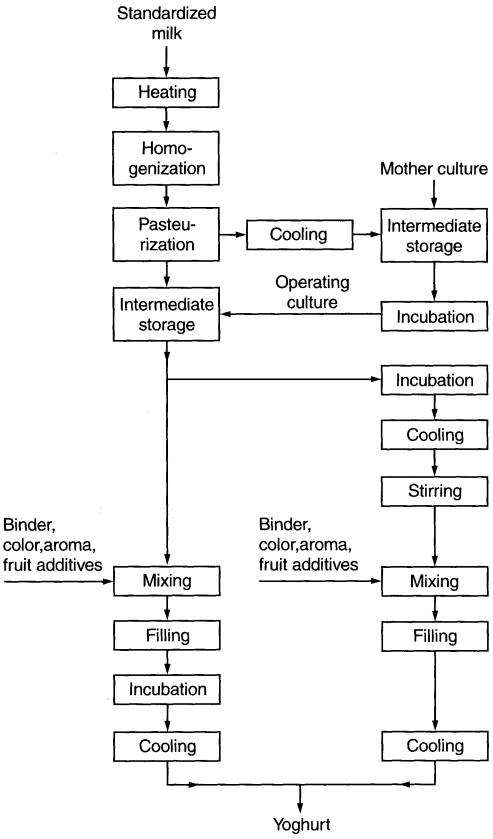
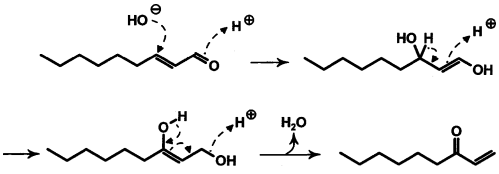


Fig. 10.21. Production of different types of yoghurt



(10.13)

10.2.1.3 Kefir and Kumiss

Kefir and kumiss are sparkling, carbonated alcoholic beverages. The microflora of kefir include *Torula* yeast (responsible for alcoholic

fermentation) and *Lactobacillus brevis, L. casei, Leuconostoc mesenteroides, Streptococcus durans, Saccharomyces delbrueckii, S. cerevisiae* and *Acetobacter aceti.* The kefir bacillus causes a buildup of "kefir grains", which resemble cauliflower heads when wet and brownish seeds when dry, and are particles of clotted milk plus the kefir organisms. Their addition to fluid milk produces kefir. Kumiss is made of mare's or goat's milk fermented by the obligatory pure kumiss culture.

Both dairy beverages are indigenous to the Caucasus and steppes of Turkestan. Kefir contains lactic acid (0.5–1.0%), noticeable amounts of alcohol (0.5–2.0%) and carbon dioxide, and some products of casein degradation resulting from proteolytic action of yeast. Normal kumiss contains 1.0–3.0% of alcohol. The production is similar to that of yoghurt.

10.2.1.4 Taette Milk

Taette (Lapp's milk) is a specially fermented, sour cow's milk product consumed in Sweden, Norway and Finland. Its thread-like, viscous structure is due to the formation of slimy substances at the low fermentation temperatures used. Mesophilic microorganisms (*Lactococcus* and *Leuconostoc spp.*) are involved in this process.

10.2.2 Cream

Milk is practically completely defatted (remaining fat content 0.03–0.06%) in hermetic, self-cleaning or hermetic/self-cleaning creaming separators. The cream products are subsequently standardized by back-mixing. Whipping cream contains at least 30% milk fat, coffee cream at least 10% and butter cream 25–82%. Cream is utilized in many ways, either by direct consumption or for production of butter and ice creams.

Whippability and stability of the whipped foam products are necessary whipping cream properties. For the best quality cream, a volume increase of at least 80% is expected and a standard cone with 100 g load must penetrate 3 cm deep in >10 s. No serum separation should occur at 18 °C after 1 h.

Fat droplets accumulate during whipping on the surface of large air bubbles which form the froth. An increased build-up of smaller bubbles tears apart the membrane of the droplet and enlarges the fat interphase area, thus resulting in gel setting of the lamella separating the individual air bubbles. Sour cream is the product of progressive lactic acid fermentation of cream.

10.2.3 Butter

Butter is a water-in-oil emulsion (w/o emulsion) made from cream by phase inversion occurring in the butter-making process. According to its manufacturing process, three types exist:

- Butter from sour cream (cultured-cream butter).
- Butter from nonsoured, sweet cream (sweet cream butter).
- Butter from sweet cream, which is soured in a subsequent step (soured butter).

Butter contains 81–85% fat, 14–16% water, 0.5–4.0% fat-free solids and 1.2% NaCl in the case of salted butter. The composition generally must meet legal standards. Butter is an emulsion with a continuous phase of liquid milk fat in which are trapped crystallized fat grains, water droplets

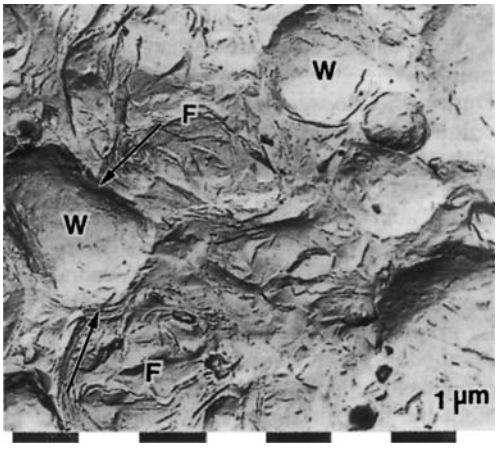


Fig. 10.22. Freeze-fracture micrograph of butter (F: fat globule, W: water droplet; according to *Juriaanse* and *Heertje*, 1988)

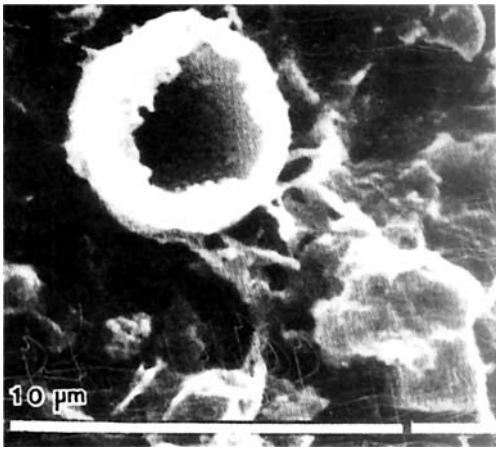


Fig. 10.23. Crystalline shell of a fat grain, as found in butter, which was obtained by eliminating the included oil; (according to *Juriaanse* and *Heertje*, 1988)

and air bubbles. A freeze-fracture micrograph of butter showing the continuous fat phase with included fat globules and water droplets is shown in Fig. 10.22. Butter consistency is determined by the ratio of free fluid fat to that of solidified fat. Due to seasonal variations in the unsaturated fatty acid content of milk fat, the solid/fluid fat ratio fluctuates at 24 °C between 1.0 in summer and 1.5 in winter. Equalization of these ratios is achieved by a preliminary cream-tempering step in a cream-ripening process, then churning and kneading the cream, which influences the extent of fluid fat inclusion into the solidified "fat grains". Figure 10.23 shows the crystalline shell of a cut fat grain, from which the liquid fat was removed during preparation.

A general idea of the most important processing steps involved in butter making is given in Fig. 10.24.

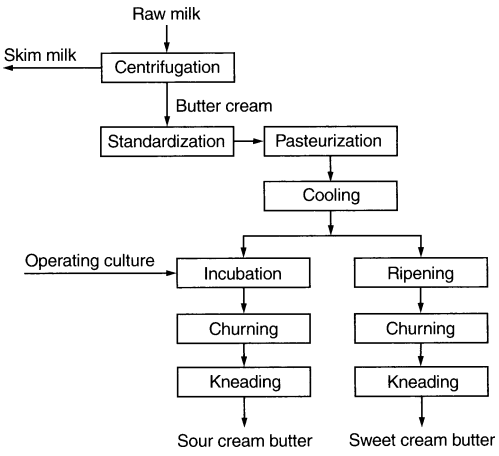


Fig. 10.24. Production of butter

10.2.3.1 Cream Separation and Treatment

Cream is separated from whole milk by high-efficiency separators (cf. 10.1.3.2 and 10.2.2). The cream, depending on the subsequent churning process, should contain 25–82% milk fat. The cream is then pasteurized at 90–110 °C.

Cream ripening and souring are the most important steps in the production of sour cream butter. The process is performed in a cream ripener or vat, with suitable mixing and temperature control. Soon after the cream has filled the ripener, a "starter culture" is added, followed by incubation for 12–24 h at 8–19 °C. The pH falls to 4.6–5.0. The "starter culture" consists of various strains of lactic acid bacteria (primarily *Lactococcus lactis subsp. lactis, Lactococus lactis subsp. cremoris, Lactococus lactis subsp. diacetylactis* and *Lactococcus lactis subsp. cremoris bv. citrovorum*). The subsequent ripening at 8–19 °C proceeds for 12 to 24 h.

The formation of fat crystals can be influenced by suitable temperature control during the cream ripening process. Consequently, the consistency of the butter can be influenced and corrected. The souring step is omitted in the production of sweet cream butter. The pasteurized cream is cooled for about 3 h at 4–6 °C to induce the crystallization of fat in the fat globules. It is then stored for about 5 h at a temperature which is 1–2 °C higher than the melting range (17–19 °C) of the low-melting milk fat fraction. As a result, a mixture of crystalline higher-melting TG and liquid low-melting TG is formed, which is easy to spread. The cream then ripens for at least 10 h at 10–14 °C.

10.2.3.2 Churning

Churning is essentially strong mechanical cream shearing which tears the membranes of the

fat globules and facilitates coalescence of the globules. The cream "breaks" and tiny granules of butter appear. Prolonged churning results in a continuous fat phase. Foam build-up is also desirable since the tiny air bubbles, with their large surface area, attract some membrane materials. Some membrane phospholipids are transferred into the aqueous phase. Buttermilk, a milky, turbid liquid, separates out initially (it is later drained off), followed by the butter granules of approx. 2 mm diameter. These granules still contain 30% of the aqueous phase. This is reduced to 15–19% by churning. The finely distributed water droplets (diameter 10 µm or less) are retained by the fat phase.

Churning is mainly carried out in stainless steel vessels of different forms which rotate nonsymmetrically. Continuously operated churns are also used with cream having a fat content of 32–38% (sour cream butter) or 40–50% (sweet cream butter). The machines are divided into churning, separation, and kneading compartments. In the churning compartment, a rotating impact wave causes butter granule formation. The separation compartment is divided into two parts. The butter is first churned further, resulting in the formation of butter granules of a larger diameter. Subsequently, the buttermilk is separated and the butter is washed, if necessary. The cooled kneading compartment consists of transport screws and kneading elements for further processing the butter. Both kneading compartments are operated under vacuum conditions to reduce the air content of the butter to less than 1%. The final salt and water content of the butter is adjusted by apportioning.

In the continuous *Alfa*-process the phase conversion is achieved in a screw-type cooler, using previously pasteurized (90%) 82% cream by repeated chilling to 8–13 °C, without the aqueous phase being separated.

The *Booser* process and the *NIZO* process allow a subsequent souring of butter from sweet cream. Both processes are of economic interest, because they yield a more aromatic sour butter and sweet buttermilk, which is a more useful by-product than sour butter-milk.

During the *Booser* process 3–4% of starter cultures are incorporated into the butter granules (water content: 13.5–14.5%) obtained from sweet cream.

Lactic acid and a flavor concentrate are obtained by separate fermentations during the first step of the *NIZO* process. In a second step they are mixed and incorporated into the butter granules from sweet cream.

Lactobacillus helveticus cultivated on whey produces the lactic acid, which is then separated by ultrafiltration and concentrated in vacuum up to about 18%. The flavor concentrate is obtained by growing starter cultures and *Lactococcus lactis subsp. diacetylactis* on skim milk of about 16% dry matter.

10.2.3.3 Packaging

After the butter is formed, it is cut by machine into rectangular blocks and is wrapped in waxed or grease-proof paper or metallic (aluminium) foil laminates (coated within with polyethylene).

10.2.3.4 Products Derived from Butter

- Melted butter consists of at least 99.3% milk fat. The aqueous phase is removed by decantation of the melted butter or by evaporation.
- Fractionated butterfat. The butter is separated by fractional crystallization into high- and low-melting fractions, and is utilized for various purposes (e. g. consistency improvement of whipping creams and butters).
- Spreadable blends with vegetable oils ("butterine").

10.2.4 Condensed Milk

Condensed milk is made from milk by the partial removal of water and addition of saccharose, if necessary (sweetened condensed milk). It is used, diluted or undiluted, like milk. Nonsweetened condensed milk is mainly available with a fat content of 7.5% or 10% and in some countries up to 15%. The solids content is 25–33%.

The production process (Fig. 10.25) starts with milk of the desired fat content. The milk is first heated, e. g., to 120 °C for 3 minutes to separate albumin, kill germs, and reduce the danger of delayed thickening. Subsequently, it is evap-

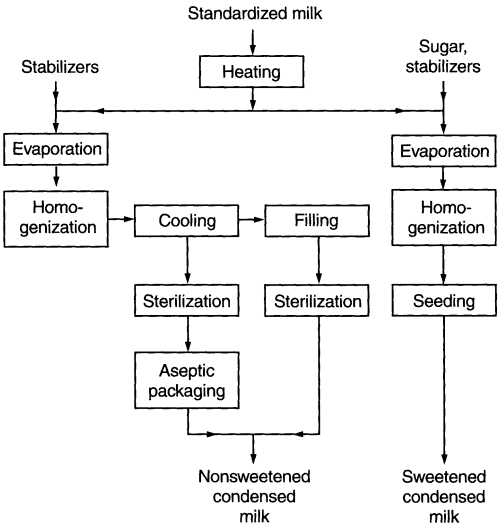


Fig. 10.25. Production of condensed milk

orated in a continuously operated vacuum evaporator at 40 to 60 °C. In comparison with previously used circulation, riser, and flat-plate evaporators, film evaporators are mainly employed today. Several units (up to seven stages) are usually connected in series, each unit being heated by the vapor from the previous stage. The temperature and pressure decrease from stage to stage. Optimal energy utilization is achieved by mechanical or thermal vapor compression. Fat separation is prevented by homogenization at 40–60 °C (12.5–25 MPa). The resulting evaporated milk, with a solid content of 24–31% or more, is homogenized, poured into lacquer (enamel)-coated cans made of white metal sheets, and is sterilized in an autoclave at 115–120 °C for 20 min. Continuous flow sterilization followed by aseptic packaging is also used. To prevent coagulation during processing and storage, Na-hydrogen carbonate, disodium phosphate and trisodium citrate are incorporated into the condensed milk. These additives have a dual effect: pH correction and adjustment of free Ca^{2+} ion concentration, both aimed at preventing casein aggregation (cf. Fig. 10.3). The additives are in the range of 0.2–0.8 g/1. They are controlled by law.

In the production of *sweetened condensed milk*, after a preheating step (short-time heating at 110–130 °C), sucrose is added to a concentration of 45–50% of the weight of the end-product. Homogenization and sterilization steps are omitted. To avoid graininess caused by lactose crystallization – the solubility limit of lactose is exceeded after sucrose addition – the condensed milk is cooled rapidly, then seeded with finely pulverized α-lactose hydrate. Seeding ensures that the lactose crystal size is 10 µm or less.

The critical quality characteristics of condensed milk are the degree of heat damage (lysine degradation), prevention of separation during the storage life, absence of coarse crystallized lactose, as well as color and taste. These criteria are influenced not only by the process management (heat treatment during evaporation and sterilization and suitable selection of the homogenization temperature and pressure), but also by the source of the milk (feed) and the producer's ability to maintain hygienic conditions.

10.2.5 Dehydrated Milk Products

Skim milk powder and whole milk powder are used either for the reconstitution of milk in countries that for climatic reasons have no dairy farming or as intermediate products for further processing into infant milk products, milk chocolate etc. The quality of these instant products depends on the durability, redissolution capacity (cold and warm), taste, microbiological characteristics, and preservation of essential constituents (proteins, vitamins) during production.

The main drying process used is spray drying. However, drum drying (with and without vacuum) and fluid-bed drying (foaming with inert gas N_2 or CO_2) are used for special purposes. Freeze drying offers no particular advantages over the less expensive spray drying process and is only of interest for special products.

Using film evaporating systems, the milk is first preconcentrated to 30–55% solids.

In drum drying, the liquid (30–40% solids) is applied in a thin layer to a heated drying cylinder (100–130 °C) and, after a defined residence time (rotation, 2–3 s), removed with a scraping knife. The liquid film can be applied in various ways. In drum drying, relatively large particles are obtained. The thermal exposure (temperature, time) is considerably higher than in spray drying, which

is consequently preferred. The solubility is poor due to the denaturation of whey proteins. The product is clearly brown owing to the *Maillard* reaction.

In spray drying, the milk concentrate (30–55% solids) is finely dispersed in the spray tower by centrifugal atomization or by nozzle atomization and dried with hot air (150–220 °C) cocurrently or countercurrently. The water content drops to 6–7% in 0.5–1 s. A further decrease to 3–4% is achieved by after drying in a vibration fluid bed with hot air (130–140 °C).

Particles with a diameter in the range of 5 to 100 µm consist of a continuous mass of amorphous lactose and other low-molecular components, which includes fat globules, casein micelles, whey proteins and usually vacuoles. When the powder absorbs water, lactose crystallizes at $a_w > 0.4$, causing agglomeration. During drying, the temperature of the particles normally does not rise above 70 °C. Therefore, the whey proteins do not denature and remain soluble. Many enzymes are still active. Storage problems are caused by the *Maillard* reaction and by fat oxidation in the case of fat-containing powders. Foam dried products can have excellent properties (aroma, solubility).

Other dehydrated dairy products, in addition to whole milk or skim milk powders, are manufactured by similar processes. Products include dehydrated malted milk powder, spray- or roller-dried creams with at least 42% fat content of their solids and a maximum 4% moisture, and butter or cream powders with 70–80% milk fat. Dehydrated buttermilk and lactic acid-soured milk are utilized as children's food.

Adaptation of infant milk product formulation to approximate mothers' milk can be achieved, for example, by addition of whey proteins, sucrose, whey or lactose, vegetable oil, vitamins and trace elements and by reduction of minerals, i. e., by a shift of the Na/K ratio.

The compositions of some dehydrated dairy products are illustrated in Table 10.27.

Table 10.27. Composition of dried milk products (%)

	1	2	3	4
Water	2.7	3	4.6	3.3
Protein	26.5	38.2	13	91.4
Fat	27.4	0.9	1.1	0.9
Lactose	37.7	49.6	73	0.2
Minerals	5.7	8.2	8.2	4.1

1: whole milk powder; 2: skim milk powder; 3: whey powder; 4: caseinate

10.2.6 Coffee Whitener

Coffee whiteners are products that are available in liquid, but more often in dried instant form. They are used like coffee cream or condensed milk. A formulation typical of these products is shown in Table 10.28. In contrast to milk products, plant fats are used in the production of coffee whiteners. Caseinates are usually the protein component. The most important process steps in the production are: preemulsification of the constituents at temperatures of up to 90 °C, high-pressure homogenization (cf. 10.1.3.4), spray drying, and instantization (cf. 10.2.5).

10.2.7 Ice Cream

Ice cream is a frozen mass which can contain whole milk, skim milk products, cream or butter, sugar, vegetable oil, egg products, fruit and fruit ingredients, coffee, cocoa, aroma substances and approved food colors. A typical formulation is 10% milk fat, 11% fat-free milk solids, 14% saccharose, 2% glucose syrup-solids, 0.3% emulsifiers, 0.3% thickener, and 62% water. The thickeners, mostly polysaccharides (cf. Table 4.15), increase the viscosity and the emulsifiers destabilize the fat globules, favoring their aggregation during the freezing process.

Table 10.28. Typical formulation of coffee whiteners

Constituent	Amount (%)
Glucose syrup	52.6
Fat	30.0
Sodium caseinate	12.0
Water	3.15
Emulsifiers	1.6
K_2HPO_4	0.6
Carrageenan	0.05
Color and aroma substances	

For the production of ice cream, the mixture of components is subjected to high-temperature short-time pasteurization (80–85 °C, 20–30 s), high-pressure homogenization (150–200 bar) and cooling to ca. 5 °C. Air is then mixed into the mixture (60–100 vol%) while it is frozen at temperatures of up to −10 °C and then hardened. The freezers used are mainly continuously working systems furnished with coolants which evaporate at −30 °C to −40 °C. The process is controlled in such a way that the core temperature of the ice cream production is ca. −18 °C.

The structural elements of ice cream are ice crystals (∼50 µm), air bubbles (60–150 µm), fat globules (<2 µm), and aggregated fat globules (5–10 µm). The fat is mostly attached to the air bubbles. The air bubbles have a three fold function: they reduce the nutritional value, soften the product, and prevent a strong cold sensation during consumption.

10.2.8 Cheese

Cheese is obtained from curdled milk by removal of whey and by curd ripening in the presence of special microflora (Table 10.29). The great abundance of cheese varieties, about 2000 worldwide, can be classified from many viewpoints, e. g., according to:

- Milk utilized (cow, goat or sheep milk).
- Curd formation (using acids, rennet extract or a combination of both).
- Texture or consistency, or water content (%) in fat-free cheese. Following the latter criterion, the more important cheese groups are (water content in %):

  Extra hard: <51%
  Hard: 49–56%
  Semihard: 54–63%
  Semisolid: 61–69%
  Soft: >67%

- Fat content (% dry matter). By this criterion, the more important groups are:

  Double cream cheese (60–85% fat);
  Cream cheese (≥50);
  Whole fat cheese (≥45);
  Fat cheese (≥40);
  Semi fat cheese (≥20);
  Skim cheese (max. 10).

Within each group, individual cheeses are characterized by aroma. A small selection of the more important cheese varieties is listed in Table 10.30. Cheese manufacturing essentially consists of curd formation and ripening (Fig. 10.26).

10.2.8.1 Curd Formation

The milk fat content is adjusted to a desired level and, when necessary, the protein content is also adjusted. Additives include calcium salts to improve protein coagulation and cheese texture, nitrates to inhibit anaerobic spore-forming microflora, and color pigments. The prepared raw or pasteurized milk is mixed at 18–50 °C in a vat with a starter culture (cf. Table 10.29) (lactic acid or propionic acid bacteria; molds, such as *Penicillium camemberti, P. candidum, P. roqueforti*; red- or yellow-smearing cultures, such as *Bacterium linens* with *cocci* and yeast). The milk coagulates into a soft, semi-solid mass, the curd, after lactic acid fermentation (sour milk cheese, pH 4.9–4.6), or by addition of rennet (sweet milk cheese, pH 6.6–6.3), or some other combination, the most common being combined acid and rennet treatment. This protein gel is cut into cubes while being heated and is then gently stirred. The whey is drained off while the retained fat-containing curd is subjected to a firming process (syneresis). The firming gets more intense as the mechanical input and the applied temperature increase. The process and the starter culture (pH) determine the curd properties. When the desired curd consistency has been achieved, curd and whey separation is accomplished either by draining off the whey or by pressing off the curd while simultaneously molding it.

New methods of cheese making aim at including the whey proteins in the curd, instead of removing them with the whey. Apart from giving higher yields (12–18%), these processes help to economize on waste water costs or elaborate whey treatments (cf. 10.2.10).

The use of ultrafiltration steps as compared with conventional cheese making is shown in Fig. 10.26. Alternatively, conventionally produced whey can be concentrated by ultrafiltration and then added to the curd or milk can be soured with starter culture and/or rennet

Table 10.29. Characteristic microflora of some types of cheese

Type of cheese	Starter cultures	Other species
Parmigiano-Reggiano	*Streptococcus thermophilus*	
	Lactobacillus helveticus	
	L. bulgaricus	
Emmental	*Lactococcus lactis*	*Propionibacterium*
	subsp. lactis	*freudenreichii*
	Lactococcus lactis	*P. freudenreichii*
	subsp. cremoris	*subsp. shermanii*
	S. thermophilus	
	Lactobacillus helveticus	
	L. bulgaricus	
Cheddar	*Lactococcus lactis*	*None*
	subsp. cremoris	
	(Lactococcus lactis	
	subsp. lactis)	
Roquefort	*Lactococcus lactis*	*Penicillium roqueforti*
	subsp. lactis	
	Lactococcus lactis	
	subsp. cremoris	
	Lactococcus lactis	
	subsp. diacetylactis	
	Leuconostoc cremoris	
Limburger	*Lactococcus lactis*	*Brevibacterium linens*
	subsp. lactis	*Micrococcus spp.*
	Lactococcus lactis	*Yeasts*
	subsp. cremoris	
Edamer, Gouda	*Lactococcus lactis*	*Brevibacterium linens*
	subsp. lactis	*Micrococcus spp.*
	Lactococcus lactis	*Yeasts*
	subsp. cremoris	
	Lactococcus lactis	
	subsp. diacetylactis	
	Leuconostoc cremoris	
Camembert, Brie	*Lactococcus lactis*	*Penicillium camemberti*
	Lactococcus lactis	
	subsp. cremoris	*P. caseicolum*
	Lactococcus lactis	*Brevibacterium linens*
	subsp. diacetylactis	*Micrococcus spp. Yeasts*

addition and then concentrated by ultrafiltration. To reduce the cost of enzymes in the casein precipitation step with chymosin (rennet or usually microbial rennet substitutes), processes using carrier-bound enzymes are being tested. Here, the enzyme reaction proceeds in the cold and precipitation occurs subsequently on heating the milk. In this way, clogging of the enzyme bed is avoided.

The individual process steps in cheese making are being increasingly mechanized and automated. The equipment used includes discontinuously operated cheesemakers (vats or tanks with stirring and cutting devices) and coagulators for continuous curd formation with subsequent fully automatic whey separation and molding.

10.2.8.2 Unripened Cheese

Unripened cheeses have a soft (quark), gelatinous (layer cheese), or grainy (cottage cheese) consis-

Table 10.30. Cheese Varieties

Unripened Cheeses (F: <10–70, T: 39–44, R: unripened)
Quark. Neuchâtel, Petit Suisse, Demi Sel, Cottage Cheese Schichtkäse (layers of different fat content) Rahm-, Doppelrahmfrischkäse, Demi Suisse, Gervais, Carré-frais, Cream Cheese Mozzarella (plastic curd by heating to >60 °C within the whey), Scamorze
Ripened Cheese
Hard Cheeses (F: 30–50, T: 58–63, R: 2–8 M)
Chester, Cheddar, Cheshire, Cantal Emmental, Alpkäse, Bergkäse, Gruyère, Herrgωrdskäse, Samsoe Gruyère (Greyerzer), l'Emmental française, Beaufort, Gruyère de Comte Parmigiano-Reggiano (granular structure, very hard, grating type), Grana, Bagozzo, Sbrinz Provolone (plastic curd by heating to >60 °C in the whey: Pasta filata), Cacciocavallo
Slicing Cheeses (F: 30–60, T: 44–57, R: 3–5 W)
Edam, Geheimratskäse, Brotkäse, Molbo, Thybo Gouda, Fynbo, Naribo Pecorino (from ewe's milk), Aunis Brinsenkäse Port Salut, St. Paulin, Esrom, Jerome, Deutscher Trappistenkäse Tilsiter, Appenzeller, Danbo, Steppenkäse, Svecia-Ost
Semi-solid slicing Cheeses (F: 30–40, T: 44–55, R: 3–5 W)
Butterkäse, Italico, Bel Paese, Klosterkäse Roquefort (from ewe's milk), Bleu d'Auvergne, Bresse Bleu, Bleu du Haut-Jura, Gorgonzola, Stracchino, Stilton, Blue Dorset, Blue Cheese, Danablue Steinbuscher Weiψlacker, Bierkäse
Soft Cheeses (F: 20–60, T 35–52, R: 2 W)
Chevre (from goat's milk), Chevret, Chevretin, Nicolin, Cacciotta, Rebbiola, Pinsgauer Käse Brie, Le Coulommiers Camembert, veritable Camembert, Petit Camembert Limburger, Backsteinkäse, Allgäuer Stangenkäse Münsterkäse, Mainauer, Mondseer, Le Munster, Gérômè Pont l'Eveque, Angelot, Maroilles Romadour, Kümmelkäse, Weinkäse, Limburger
Low-fat Cheeses (F: <10, T: 35, R: 1–2 W)
Harzer Käse, Mainzer Käse (ripened with *Bact. linens*, different cocci and yeasts) Handkäse, Korbkäse, Stangenkäse, Spitzkäse (ripened with *Bact. linens*, different cocci and yeasts, or with *Penicillium camemberti*), Gamelost
Cooking cheese (from Cottage Cheese by heating with emulsifying agents, F: <10 60)

[a] Related types are grouped together. For the classes average values are given for
- fat content in the dry matter: F (%)
- dry matter: T(%)
- ripening time: R in months (M) or weeks (W).

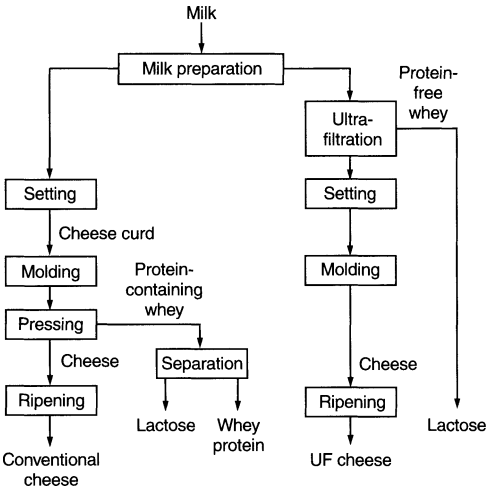


Fig. 10.26. Cheese making (conventional or with ultrafiltration)

tency. In the production of quark, the whey is usually separated after souring. Cottage cheese is generally produced in continuously operated coagulators with special temperature regulation. After whey separation via a filter band, the curd grain can be washed in a screw vat, cooled, and dried via another drying band.

10.2.8.3 Ripening

The molded cheese mass is placed in a salt bath for some time, dried, and then left to ripen in air-conditioned rooms. Ripening or curing is dependent on cheese mass composition, particularly the water content, the microflora and the external conditions, such as temperature and humidity in the curing rooms.

The ripening of soft cheeses proceeds inwards, so in the early stages there is a ripened rind and an unripe inner core. This nonuniform ripening is due to the high whey content which causes increased formation of lactic acid and a pH drop at the start of ripening. In the rind, special molds that grow more favorably at higher pH values contribute to a pH increase by decarboxylating amino acids.

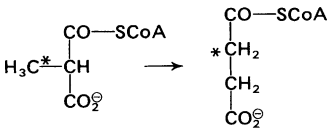
Ripening in hard cheeses occurs uniformly throughout the whole cheese mass. Rind formation is the result of surface drying, so it can be avoided by packaging the cheese mass in suitable plastic foils before curing commences. The duration of curing varies and lasts several days for soft cheeses and up to several months or even a couple of years for hard cheeses. The yield per 100 kg fluid milk is 8 kg for hard cheeses and up to 12 kg for soft cheeses.

All cheese ingredients are degraded biochemically to varying extents during curing.

Lactose is degraded to lactic acid by homofermentation. In cheddar cheese, for example, the pH drops from 6.55 to 5.15 from the addition of the starter culture to the end of mold pressing. In the presence of propionic acid bacteria (as in the case of Emmental cheese), lactic acid is converted further to propionic and acetic acids and CO_2, according to the reaction:

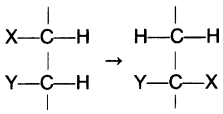
$$3\,CH_3CHOHCOOH \rightarrow 2\,CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O \qquad (10.14)$$

The ratio of propionic to acetic acid is influenced by the redox potential of the cheese, and in the presence of nitrates, for example, the ratio is lower. Propionic acid fermentation is shown in Fig. 10.27. The crucial step is the reversible rearrangement of succinyl-CoA into methylmalonyl-CoA:



(10.15)

The catalysis is mediated by adenosyl-B_{12}, which is a coenzyme for transformations of the general type:



(10.16)

Based on a study of a coenzyme B_{12}-analogue, it is obvious that a nonclassical carbanion mechanism is involved:

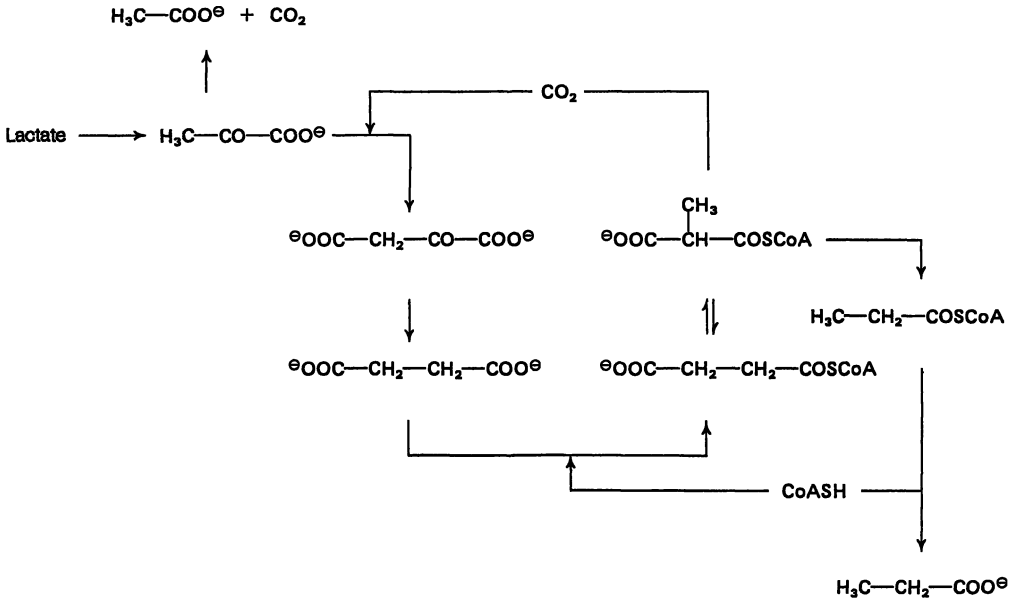
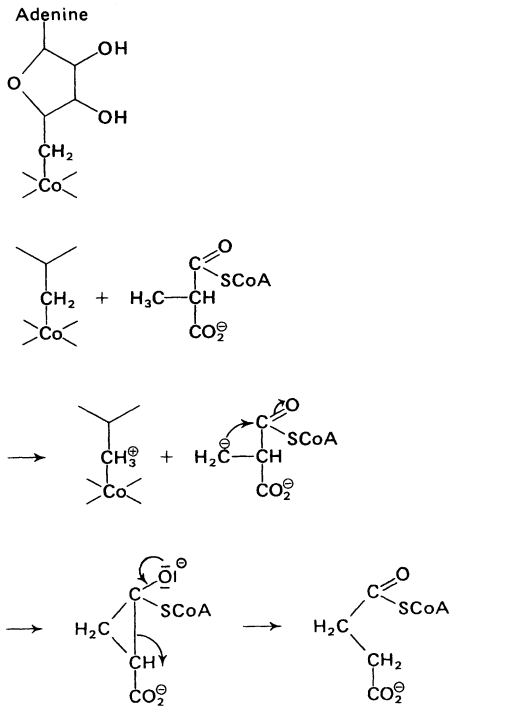


Fig. 10.27. Scheme for propionic acid fermentation



(10.17)

The mode and extent of milk *fat* degradation depend on the microflora involved in cheese ripening. In most types of cheese, as little lipolysis as possible is a prerequisite for good aroma. Exceptions are varieties like Roquefort, Gorgonzola, and Stilton, that are characterized by a marked degradation of fat.

Lipolysis is strongly enhanced by homogenization of the milk (Fig. 10.28). The release of fat-

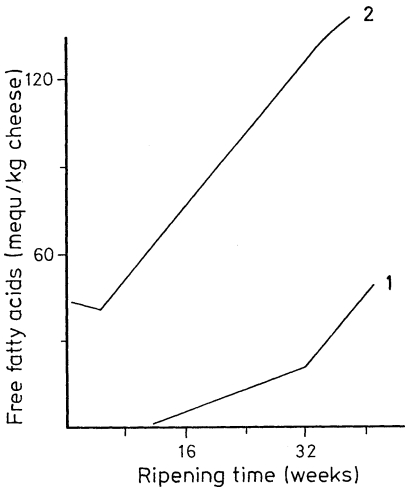


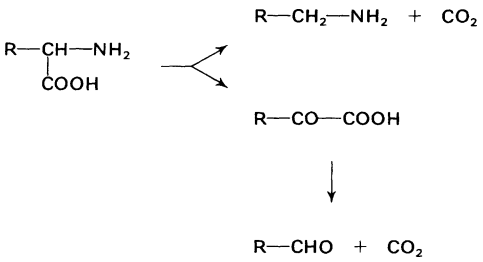
Fig. 10.28. Lipolysis during ripening of blue cheese: *1* untreated milk, *2* homogenized milk

ty acids, especially those that affect cheese aroma, depends on the specificity of the lipases (Table 10.31). In addition to free fatty acids, 2-alkanones and 2-alkanols are formed as by-products of the β-oxidation of the fatty acids (cf. 3.7.5).

Molds, particularly *Penicillium roqueforti*, utilize β-ketoacyl-CoA deacylase (thiohydrolase) and β-ketoacid decarboxylase to provide the compounds typical for the aroma of semi-soft cheeses e. g., the blue-veined cheese (Roquefort, Stilton, Gorgonzola, cf. Table 10.32).

Protein degradation to amino acids occurs through peptides as intermediary products. Depending on the cheese variety, 20–40% of casein is transformed into soluble protein derivatives, of which 5–15% are amino acids. A pH range of 3–6 is optimum for the activity of peptidases from *Penicillium roqueforti.* Proteolysis is strongly influenced by the water and salt content of the cheese. The amino acid content is 2.8–9% of the cheese solids. Of the amino acids released, glutamic acid is of special importance to cheese taste (cf. 10.3.5). Ripening defects can produce bitter-tasting peptides.

The amino acids are transformed further. In early stages of cheese ripening, at a lower pH, they are decarboxylated to amines. In later stages, at a higher pH, oxidation reactions prevail:



(10.18)

Proteolysis contributes not only to aroma formation, but it affects cheese texture. In overripening of soft cheese, proteolysis can proceed almost to liquefaction of the entire cheese mass.

The progress of proteolysis can be followed by electrophoretic and chromatographic methods, e. g., via the peptide pattern obtained with the help of RP-HPLC (Fig. 10.29) and via changes in concentration of individual peptides which correspond to certain casein sequences (Table 10.33) and can serve as an indicator of the degree of cheese ripening.

The decarboxylation of amino acids (name in brackets) leads to the biogenic amines phenylethylamine (phenylalanine), tyramine (tyrosine), tryptamine (tryptophan), histamine (histidine), putrescine (ornithine) and cadaverine (lysine). The content of these compounds in some types of cheese is presented in Table 10.34. These values can fluctuate greatly depending on the degree of ripening. On average, 350–500 µmol per person per day are consumed. Apart from cheese,

Table 10.31. Substrate specificity of a lipase from *Penicillium roqueforti*

Substrate	Hydrolysis (V_{rel})
Tributyrin	100
Tripropionin	25
Tricaprylin	75
Tricaprin	50
Triolein	15

Table 10.32. 2-Alkanones in blue cheese

2-Alkanone n[a]	mg/100 g cheese (dry matter)
3	0.5–0.8
5	1.4–4.1
7	3.8–8.0
9	4.4–17.6
11	1.2–5.9

[a] Number of C-atoms.

Table 10.33. Amino acid sequences of some small peptides from Cheddar cheese

Peptide[a]	Sequence	Corresponding casein sequence	
30	A P F P E	α_{s1}B	26–30[b]
37	D K I (H) P F	βA^2	47–52
39	L P Q E (V L)	α_{s1}B	11–16
46	L Q D K I (H) P (F)	βA^2	45–52
58	Y P F P G P I P N	βA^2	60–68
60	A P F P E (V F)	α_{s1}B	26–32[b]

[a] Numbering cf. Fig. 10.29.

[b] In the literature, Q represents position 30 of α_{s1}-casein B, and E the corresponding position of the precursor protein. () Added on the basis of the amino acid composition.

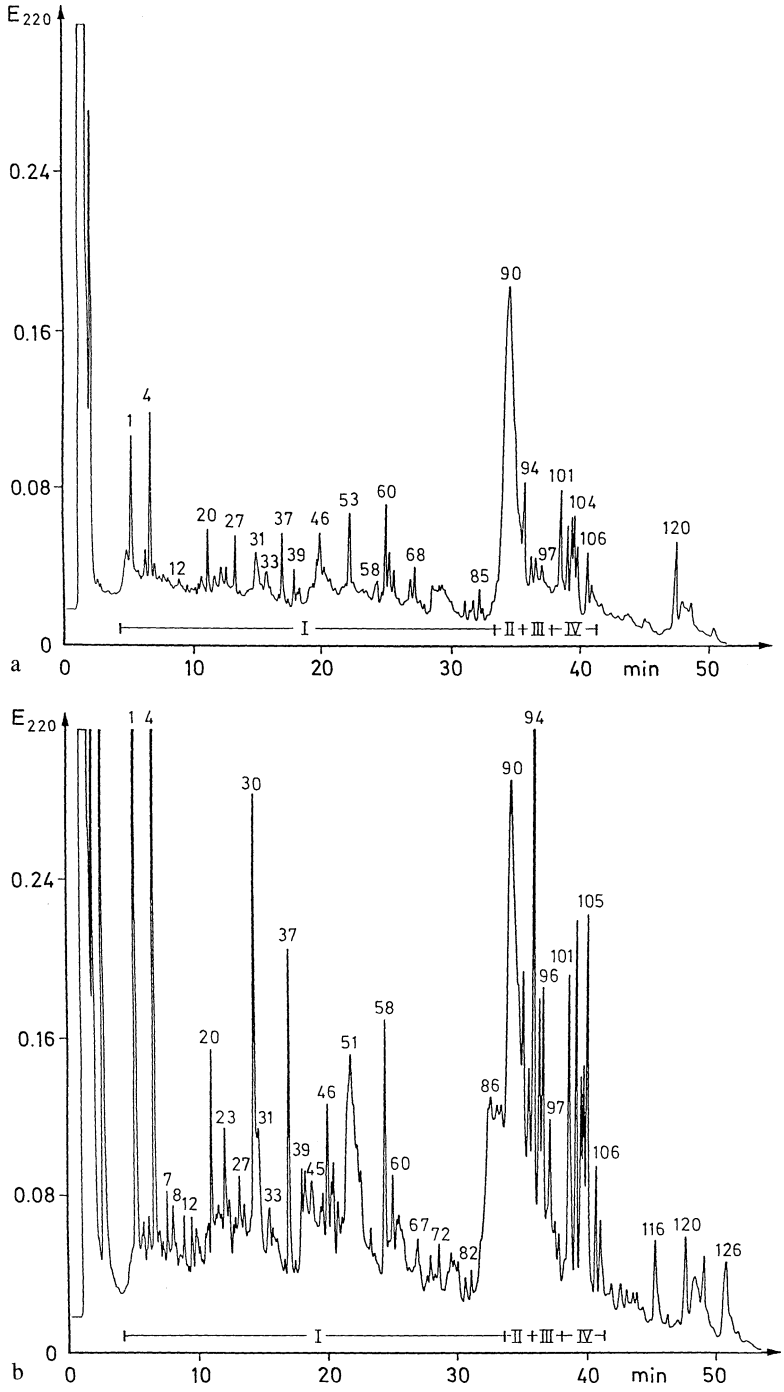


Fig. 10.29. RP-HPLC of the pH 4.6-soluble fraction of a citrate extract of Cheddar cheese after 3 **(a)** and 24 **(b)** weeks; ripening at 10 °C (according to *Kaiser* et al., 1992)

Table 10.34. Biogenic amines in cheese (mg/100 g)

Cheese	Phenylethyl-amine	Tyramine	Tryptamine	Histamine	Putrescine[a]	Cadaverine[b]
Cheddar	0–30	6–112	0–0.2	2.4–140	0–100	0–88
Emmentaler	0–23.4	3.3–40	0–1.3	0.4–250	0–15	0–8
Gruyere		6.4–9.9		0–20	0.5	2.5
Parmesan		0.4–2.9		0–58		
Provolone					1–20	2–20
Edamer	0–1.3		0–0.4	1.4–6.5		0.5–9.4
Gouda		0–110		3.5–18	2–20	2.5
Tilsiter	0–14.8	0–78	0–7.1	0–95.3	0–31.3	0–31.8
Gorgonzola					0–75	0–430
Roquefort		2.7–110	0–160	1–16.8	1.5–3.3	7.1–9.3
Camembert		2–200	2	0–48	0.7–3.3	1.2–3.7

[a] Butane-1,4-diamine
[b] Pentane-1,5-diamine

fruits (cf. 18.1.4.2.1) and meat (cf. 12.3.5) are important sources.

10.2.8.4 Processed Cheese

Processed (or melted) cheese is made from natural, very hard grating or hard cheeses by shredding and then heating the shreds to 75–95 °C in the presence of 2–3% melting salts (lactate, citrate, phosphate) and, when required, utilizing other ingredients, such as milk powder, cream, aromas, seasonings and vegetable and/or meat products. The cheese can be spreadable or made firm and cut as desired. The shelf life of processed cheese is long due to thermal killing of microflora.

The heating process is carried out batchwise by steam injection in a double-walled pressure vessel equipped with a mixer, usually under a slight vacuum. Continuous processes are conducted in double-walled cylinders with agitator shafts.

10.2.8.5 Imitation Cheese

Imitation cheese (analogue cheese) is mainly found in North America. They are made of protein (mostly milk protein), fat (mostly hardened vegetable fat), water, and stabilizers by using processed cheese technology. A typical formulation is shown in Table 10.35.

Table 10.35. Typical formulation of imitation cheese (Mozarella type)

Component	Amount (%)
Water	51.1
Ca/Na caseinate	26.0
Vegetable oil (partially hydrogenated)	8.0
Glucono-δ-lactone	2.8
Salt	2.0
Color and aroma substances	

10.2.9 Casein, Caseinates, Coprecipitate

The production of casein, caseinates, and coprecipitate is shown schematically in Fig. 10.30.

Coagulation and separation of casein from milk is possible by souring the milk by lactic acid fermentation, or by adding acids such as HCl, H_2SO_4, lactic acid or H_3PO_4. Another way to achieve coagulation is to add proteinase enzymes, such as chymosin and pepsin. The acid coagulation is achieved at 35–50 °C and pH 4.2–4.6 (isoelectric point of casein is pH 4.6–4.7). Casein precipitates out as coarse grains and is usually separated in sedimentation centrifuges, washed, and dried (whirlwind drier). The enzymatic process involves heating to 65 °C after precipitation in whey.

Increasing the level of Ca^{2+} ions (addition to milk of 0.24% $CaCl_2$) causes casein and whey proteins to coagulate when the temperature is

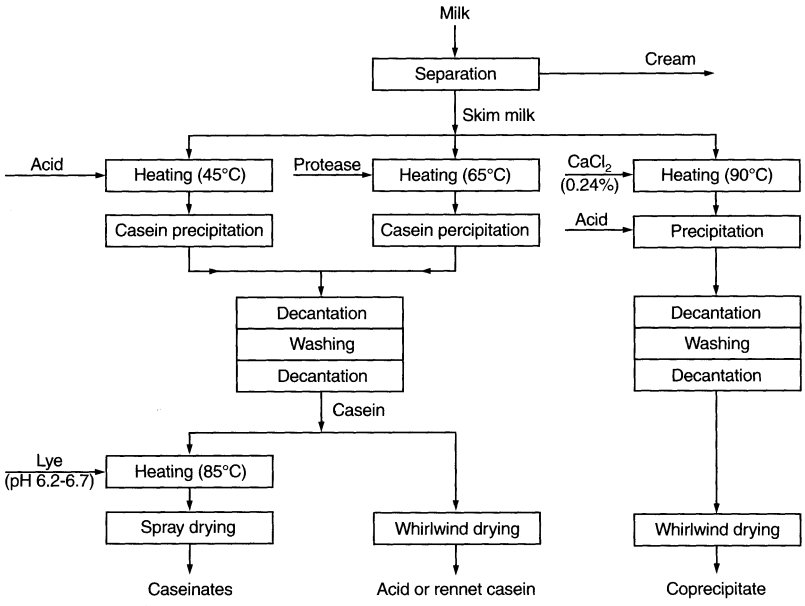


Fig. 10.30. Production of casein, caseinates, and coprecipitate

at 90 °C. Joint coagulation of proteins can also be achieved by first heat-denaturing the whey proteins, then acidifying the milk. Washing followed by drying of the curd gives a coprecipitate which contains up to 96% of the total proteins of the milk. When casein dispersions, 20–25%, are treated with alkali [NaOH or $Ca(OH)_2$, alkali or alkaline-earth carbonates or citrates] at 80–90 °C and pH 6.2–6.7, and then the solubilized product is spray-dried, a soluble or readily dispersable casein product is obtained (caseinate, disintegrated milk protein).

Caseins and whey proteins are also concentrated by ultrafiltration and reverse osmosis. Since the molar masses of the whey proteins and casein micelles are in the range of 10^3–10^4 and 10^7–10^9 respectively, membranes with a pore diameter of 5–50 nm are suitable for the separation of these proteins.

Casein and caseinate are utilized as food and also have nonfood uses. In food manufacturing they are used for protein enrichment and/or to achieve stabilization of some physical properties of processed meats, baked products, candies, cereal products, ice creams, whipping creams, coffee whiteners, and some dietetic food products and drugs.

The nonfood uses involve wide application of casein/caseinate as a sizing (coating) for better quality papers (for books and journals, with a surface suitable for fine printing), in glue manufacturing, as a type of waterproof glue (alkali caseinate with calcium components as binder); in the textile industry (dye fixing, water-repellent impregnations); and for casein paints and production of some plastics (knobs, piano keyboards, etc.).

10.2.10 Whey Products

Whey accumulates in considerable amounts in the production of cheese and casein.

The composition of whey and whey products is presented in Table 10.36. Whey and whey products are used in animal feed, dietetic foods (infant food), bread, confectionery, candies, and beverages.

10.2.10.1 Whey Powder

In dairy farming, two process variants are applied for the drying of whey:

Table 10.36. Protein, lactose and mineral contents of whey products[a]

Product	DM[b] (%)	Protein (%)	Lactose (%)	Minerals (%)
Skim milk	9.0	36	53	7
Whey (from coagulating with rennet)	6.0–6.4	13	75	8
Whey (from coagulating with acid)	5.8–6.2	12	67	14
Demineralized whey powder		12–13	85	1–2
Whey protein powder[c]				
I		47	44	9
II		74	20	6

[a] Average values are expressed as % of dry matter.
[b] Dry matter.
[c] After one (I) and two (II) ultrafiltrations.

- Preliminary concentration of the whey to 50–55% dry matter in falling-film evaporating systems (thermal or mechanical vapor compression), followed by spray drying (one step or two step with subsequent vibraton fluid bed).
- Preliminary concentration of the whey to 21–25% dry matter by reverse osmosis (hyperfiltration), followed by concentration to 50–55% dry matter via falling-film evaporators and spray drying.

The composition of whey powder is presented in Tables 10.27 and 10.36.

10.2.10.2 Demineralized Whey Powder

In the applications of whey powder, the minerals can interfere with the taste. The production of demineralized whey powder proceeds via ion exchange or, preferentially, electrodialysis (1.5–4.5V/cell; current density 5–20 mA/cm^2 membrane area, Fig. 10.31). The course of demineralization is shown in Fig. 10.32.

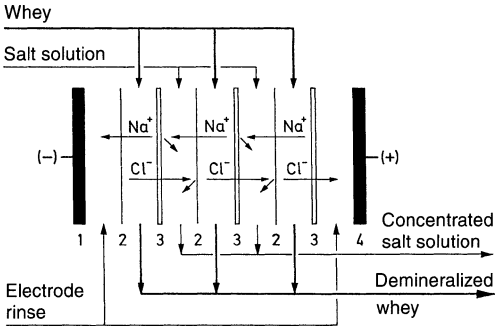


Fig. 10.31. Principle of electrodialysis of whey. *1* cathode, *2* cation membrane, *3* anion membrane, *4* anode

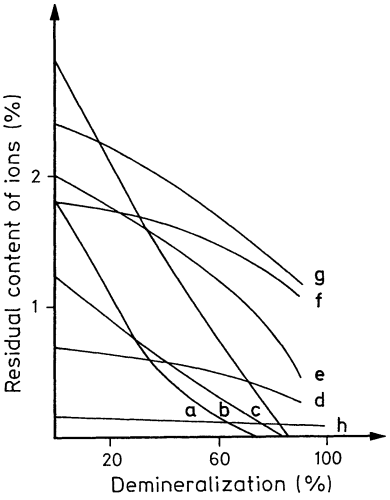


Fig. 10.32. Whey demineralization. Ions of *a* chloride, *b* sodium, *c* potassium, *d* calcium, *e* phosphate, *f* lactate, *g* citrate, and *h* magnesium

10.2.10.3 Partially Desugared Whey Protein Concentrates

In the ultrafiltration of whey, protein concentrates depleted of lactose to various extents are obtained, depending on the number of stages and amount of wash water. Another, less gentle method involves the heating of whey (95 °C, 3–4 min) by direct steam injection, followed by precipitation of the denatured proteins at pH 4.5, separation in a sedimentation centrifuge (2000–4000 min^{-1}), and drying.

10.2.10.4 Hydrolyzed Whey Syrups

The production of sweet whey syrups is becoming increasingly important due to the use of carrier-bound lactase (β-galactosidase, EC. 3.2.1.23). In these syrups, lactose is hydrolyzed to glucose and galactose. Concentration to 60–75% solids is achieved by evaporation.

10.2.11 Lactose

For lactose production the whey is evaporated to 55–65% solid content, and the concentrate is then seeded and cooled slowly to induce sugar crystallization. The raw lactose (food quality) is recrystallized to yield a raffinade (pharmaceutical-grade lactose). Lactose is used in manufacturing of drugs (tablet filler), dietetic food products, baked products, dehydrated foods, cocoa products, beverages and ice creams.

10.2.12 Cholesterol-Reduced Milk and Milk Products

In the production of milk products with a reduced cholesterol content, more than 90% of the cholesterol is removed from water-free milk fat by extraction with supercritical carbon dioxide or by steam distillation. The fat is then recombined with skim milk to give low-cholesterol milk, which is used to make the usual milk products. The extent of cholesterol reduction in a series of products is listed in Table 10.37.

Recombined milk does not have the same properties as the original milk because, e. g., the membrane composition of the fat globule changes in the process. Cheese made from milk of this type can exhibit texture defects. Since skim milk with a fat content of 0.2% still contains about 18 mg/l of cholesterol, skim milk must also be freed of cholesterol for the production of cholesterol-free products.

Table 10.37. Effects of a 90% reduction of cholesterol in butter oil on the cholesterol content of recombined milk and its products

Food	Fat (%)	Cholesterol (mg/kg)	
		I[a]	II[a]
Whole milk	3.3	135	26
Butter	81	2400	300
Yoghurt	3.5	124	26
Ice cream	10.8	450	41
Cottage cheese	4.6	150	12
Mozzarella	21.6	786	68
Brie	20.8	1000	75
Camembert	24.6	714	57
Roquefort	30.6	929	107
Cheddar	33.1	1071	114

[a] Product before (I) and after (II) cholesterol reduction.

10.3 Aroma of Milk and Dairy Products

10.3.1 Milk, Cream

Raw or gently pasteurized milk has a mild but characteristic taste.

In the AEDA of UHT milk (Table 10.38), δ-decalactone, which contributes to the aroma of butter (Table 10.40) as well as unripened and ripened cheese (cf. 10.3.5), is the predominant aroma substance. Apart from other lactones, 2-acetyl-1-pyrroline, methional, 2-acetyl-2-thioazoline and 4,5-epoxy-2-decenal are among the identified aroma substances.

A higher thermal exposure of milk, e. g., by sterilization, allows the accumulation of *Maillard* products, such as methylpropanal, 2- and 3-methyl butanal and 4-hydroxy-2,5-dimethyl-3(2H)-furanone.

10.3.2 Condensed Milk, Dried Milk Products

During the concentration and drying of milk, reactions that are similar to those described for heat-treated milk (cf. 10.1.3.5 and 10.3.1) occur, but to a greater extent. Therefore, like the aroma of UHT milk (cf. 10.3.1 and Table 10.38), the aroma of condensed milk is also caused by *Maillard* reaction products. The stale flavor that appears when condensed milk is stored for longer periods is due especially to the presence of the degradation product of tryptophan, o-aminoacetophenone, which is aroma active in concentrations $\geq 1\,\mu g/kg$. A rubbery aroma defect results from higher concentrations of benzothiazole.

Table 10.38. Potent aroma substances of UHT milk[a]. Result of an AEDA

Compound	Odor quality	FD Factor
2-Acetyl-1-pyrroline	Rusty	8
(Z)-4-Nonenal	Fatty	1
Methional	Boiled potatoes	8
2,3-Diethyl-5-methylpyrazine	Earthy	1
Unknown	Fatty, cardboard	1
Butyric acid	Sweaty	4
Unknown	Mint	2
2-Acetyl-2-thiazoline	Rusty	8
Caproic acid	Sweaty	2
d-Octalactone	Coconut	16
trans-4,5-Epoxy-(E)-2-decenal	Metallic	16
Caprylic acid	Sweaty	1
δ-Nonalactone	Coconut	1
Unknown	Musty	2
δ-Decalactone	Coconut	128
Unknown	Musty	8
Capric acid	Sweaty	2
Unknown	Coconut	1
γ-Dodecalactone	Coconut	16
γ-(Z)-6-Dodecenol-actone	Coconut	16
Unknown	Woody	8
Vanillin	Vanilla	16

[a] In bottles

Table 10.39. Aroma substances in raw cream (I), whipped raw cream (II) and in whipped pasteurized raw cream (III)

No.	Aroma substance	Concentration (μg/kg)		
		I	II	III
1	Butyric acid	4400	8000	2000
2	Caprylic acid	4200	7500	1800
3	δ-Dedecalactone	1100	1400	1200
4	δ-Decalactone	300	300	250
5	γ-Dodecalactone	63	99	63
6	δ-Octalactone	28	37	26
7	3-Methylbutyric acid	18	18	17
8	(Z)-6-Dodecen-g-lactone	7.5	10	9.2
9	3-Methylindol	3.4	3.1	3.4
10	(Z)-3-Hexenal	1.6	3.3	7.7
11	(E)-2-Nonenal	1.3	1.7	0.8
12	trans-4,5-Epoxy-(E)-2-decenal	1	0.97	0.29
13	2-Phenylethanol	0.57	0.58	0.51
14	(E)-2-Ddodecenal	0.37	0.37	0.4
15	1-Octen-3-one	0.33	0.19	0.11
16	(E,Z)-2,6-Nonadienal	0.11	0.2	1.4
17	2-Aminoacetophenone	0.13	0.15	0.13
18	1-Hexen-3-one	0.1	0.1	0.21
19	Methional	0.07	0.06	0.07
20	2-Acetyl-1-pyrroline	0.03	0.05	0.07
21	2-Acetyl-2-thiazoline	n.d.	n.d.	0.06
22	Methanthiol	n.d.	n.a.	27
23	Dimethylsulfide	10	n.a.	13

[a] Cream (fat content: 30%); n.d.: not detected, n.a.: not analyzed

The content of free butyric and caprylic acid as well as (Z)-3-hexenal rises when cream is whipped (Table 10.39). Pasteurization results in the formation of 2-acetyl-2-thiazoline in whipped cream and the content of (E,Z)-2,6-nonadienal is greatly increased. A model corresponding to Table 10.39 (without No. 12, 14, 17 and 20) approaches the aroma of whipped pasteurized cream and reproduces especially the "creamy" note.

Maillard reaction products are also characteristic of the aroma of milk powder. The development of aroma defects during the storage of whole milk powder is due to products of lipid peroxidation, e. g., (Z)- and (E)-2-nonenal.

10.3.3 Sour Milk Products, Yoghurt

Metabolic products of lactic acid bacteria, such as diacetyl, ethanal, dimethylsulfide, acetic acid and lactic acid contribute to this aroma. Carbon dioxide also appears to be important. In good sour milk products, the concentration ratio of diacetyl/ethanal should be ca. 4. At values of ≤ 3, a green taste appears, which is to be regarded as an aroma defect. Diacetyl is formed from citrate (Fig. 10.33). The conversion of acetolactate to diacetyl is disputed. It should occur spontaneously or be catalyzed by an α-acetolactate oxidase.

Ethanal greatly contributes to the aroma of yoghurt. Concentrations of 13–16 μg/kg are characteristic of good products.

10.3.4 Butter

Only the three compounds listed in Table 10.40 make an appreciable contribution to the aroma of butter. A comparison of the aroma profiles of five samples of butter (Table 10.41) with the results of a quantitative analysis (Table 10.40) show that

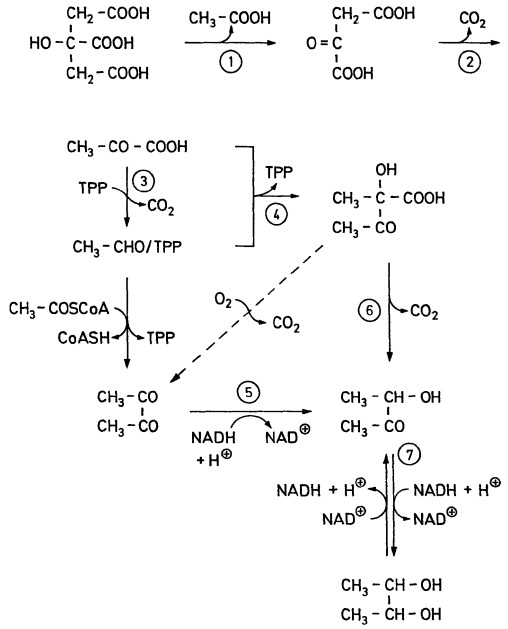


Fig. 10.33. Formation of diacetyl and butanediol from citrate by Streptococci. *1* citratase, *2* oxaloacetate decarboxylase, *3* pyruvate decarboxylase, *4* α-acetolactate synthase, *5* diacetyl reductase, *6* α-acetolactate decarboxylase, *7* 2,3-butanediol dehydrogenase

Table 10.40. Concentrations of the key aroma substances in five samples of butter[a]

Aroma substance	Concentration (mg/kg) in sample number				
	1	2	3	4	5
Diacetyl	0.62	0.34	0.11	0.32	<0.01
(R)-δ-Decalactone	5.0	4.91	3.06	2.15	3.8
Butyric acid	4.48	3.63	2.66	94.5	2.48

[a] The aroma profiles of the samples are presented in Table 10.41.

the concentrations of these three odorants, which are present in Samples 1 and 2, produce an intensive butter aroma. In Samples 3 and 4, especially the diacetyl content is too low and in Sample 4, the excessively high butyric acid concentration stimulates a rancid aroma defect. Lactic acid is primarily involved in the taste of sour cream butter.

If butter contains lipases, fatty acids are released on storage. Above certain limiting concentrations

Table 10.41. Aroma profile of samples of butter

No.	Sample	Odor quality	Intensity[a]
1	Sour cream	Buttery, creamy, sweet	3
2	Sour cream	Buttery, creamy	2–3
3	Sour cream	Slightly buttery, mild, sour	1–2
4	Sour cream	Rancid, like butyric acid	3
5	Sweet cream	Mild, somewhat sour	1

[a] Evaluation: 1, weak; 2, medium; 3, strong.

(cf. 3.2.1.1), these fatty acids cause a rancid off-flavor.

Rancid, soapy aroma defects, which occur in butter samples with very low concentrations of free fatty acids, can be due to contamination with anionic detergents (sodium dodecyl sulfate, sodium dodecyl benzosulfonate). Detergents of this type are used to disinfect the udder and the milking machine.

10.3.5 Cheese

The aroma profile of unripened cheese, e. g., Mozzarella, consists of butter-like, sweetish, salty and sour notes produced by l, δ-decalactone, NaCl and lactic acid. The characteristic odor and taste of the type of cheese are formed during ripening, whereby the composition of the microflora and the storage conditions (temperature, air humidity, time) have the greatest influence. For a soft cheese (Camembert) and a hard cheese (Emmentaler), the compounds mainly responsible for the odor and taste in the ripened product will be discussed here.

The butter-like note of unripened cheese can still be detected in Camembert and Emmentaler, but the intensity is lower, because other aroma substances formed during ripening become evident. Thus, Camembert also has mushroom-like, sulfurous and flowery notes and Emmentaler, nutty, sweet and fruity notes. In comparison with unripened cheese, the taste profile is extended to include a glutamate note and in the case of Emmentaler, an additional and characteristic sour/pungent impression.

Among the odorants in Camembert (Table 10.42), 1-octen-3-ol is responsible for the mushroom-like note, which should be intensified by 1-octen-3-one. Although its concentration is only 2.1 µg/kg, it is aroma-active in Camembert because its odor threshold is 100 times lower than that of the alcohol. Methanethiol, methional, dimethylsulfide and methylene-bis(methylsulfide) produce the sulfurous and phenylethylacetate produces the flowery note. The relatively high concentration of glutamate (Table 10.42) is noticeable in the taste. Methylketones contribute to the typical aroma of blue cheese (Roquefort). It is unknown which additional compounds are important.

Table 10.43 shows the odorants and taste compounds of Emmental. The high concentration of more than 6 g/kg of propionic acid produces the

Table 10.42. Odorants and taste compounds of Camembert[a]

Compound/Ion	Concentration[b] (mg/kg)
Odorant	
Diacetyl	0.074–0.110
3-Methylbutanal	0.094–0.142
Methional	0.027–0.125
1-Octen-3-ol	0.075–0.130
1-Octen-3-one	0.0021
Phenethylacetate	0.250–0.320
2-Undecanone	0.180–0.700
δ-Decalactone	0.910–1.08
Methanthiol	0.260–0.275
Dimethylsulfide	0.250–0.410
Acetaldehyde	0.015–0.025
Hexanal	0.124–0.144
Dimethyltrisulfide	0.008–0.010
Methylen-bis(methylsulfide)	0.250–0.360
2-Acetyl-1-pyrroline	0.001–0.003
2-Phenylethanol	0.130–0.137
Taste compound	
Acetic acid	59–92
Butyric acid	122–130
3-Methylbutyric acid	3.4–4.5
Caprylic acid	62–70
Glutamic acid[c]	2690–4381
Lactic acid	88–174
Succinic acid[c]	535–892
Ammonia[c]	448–632
Sodium[c]	12,190–13,570
Potassium[c]	665–743
Calcium[c]	761–802
Magnesium[c]	61–97
Chloride[c]	12,053–14,180
Phosphate[c]	1330–1425

[a] Fat: 45% solids, Water: 52%.
[b] Reference: fresh weight.
[c] Concentration in aqueous extract from 1 kg cheese.

Table 10.43. Oderants and taste compounds of Emmentaler[a]

Compound/Ion	Concentration[b]
Odorant (µg/kg)	
Diacetyl	431
2-Methylbutanal	181
3-Methylbutanal	145
Butyric acid ethyl ester	27
3-Methylbutyric acid ethyl ester	0.40
2-Heptanone	522
Dimethyltrisulfide	0.11
Methional	67
Caproic acid ethyl ester	51
1-Octen-3-one	0.06
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F)	1186
5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone (EHM3F)	253
2-*sec*-Butyl-3-methoxypyrazine	0.07
Skatole	47
δ-Decalactone	3751
Taste compound (mg/kg)	
Acetic acid	3830
Propionic acid	6750
Butyric acid	70
3-Methylbutyric acid	20
Glutamic acid[c]	5380
Lactic acid[c]	9150
Succinic acid[c]	1320
Ammonia[c]	560
Sodium[c]	5150
Potassium[c]	1280
Calcium[c]	6650
Magnesium[c]	680
Chloride[c]	3730
Phosphate[c]	10570

[a] Ripening time 3 months.
[b] Reference: dry matter (water content: 36%, fat: 50% solids).
[c] Concentration in aqueous extract from 1 kg cheese.

characteristic sour/pungent taste, which is intensified by lactic acid. The two furanones HD3F and EHM3F probably contribute to the nutty note. Model experiments show that lactic acid bacteria (*Lactobacillus helveticus, Lactobacillus delbrueckii*) are involved in the formation of HD3F.

At the Emmentaler pH of 5.6, magnesium (threshold value: 3.5 mmol/kg) and calcium propionate (7.1 mmol/kg) taste sweet. Consequently, it is assumed that these propionates contribute to the sweet note. On the other hand, glutamic acid is an important taste substance, which has the additional function of neutralizing the bitter taste of amino acids and peptides. Only if the concentrations of these constituents climb too high on longer ripening of Emmentaler, the effect of glutamic acid is no longer sufficient and the bitter taste appears. An off-flavor can also be formed if there is a greater increase in the fatty acids 4:0–12:0.

The caseins are increasingly degraded during longer ripening. Water-soluble peptides and amino acids are formed which bind a part of the ions. Thus, when chewing a cheese ripened for a long time, the water-soluble portion of the ions increases, possibly causing an intensification of the salty taste.

It is probable that not only peptides, but also other amides are responsible for the bitter taste of cheese. For example, the presence of bitter N-isobutyl acetamide has been detected in Camembert cheese.

10.3.6 Aroma Defects

As already indicated, aroma defects can arise in milk and milk products either by absorption of aroma substances from the surroundings or by formation of aroma substances via thermal and enzymatic reactions.

Exogenous aroma substances from the feed or cowshed air enter the milk primarily via the respiratory or digestive tract of the cow. Direct absorption apparently plays only a minor role. Metabolic disorders of the cow can cause aroma defects, e. g., the acetone content of milk is increased in ketosis.

The oxidation of lipids is involved in the endogenous formation of aroma defects. While very low concentrations of certain carbonyl compounds, e. g., (Z)-4-heptenal (1 µg/kg), 1-octen-3-one, and hexanal, appear to contribute to the full creamy taste, increased concentrations of these and other compounds produce cardboard-like, metallic, and green aroma notes. In butter, for instance, the phospholipids of the fat globule membrane are especially susceptible to oxidation. The subsequent products get distributed in the entire fat fraction and cause taste defects which range from metallic to fatty and from fishy to tallowy.

Light can cause the degradation of methionine to 3-methylthiopropanal via riboflavin as sensitizer. Together with other sulfides and methanethiol, this sulfur compound produces the aroma defect of milk and milk products called “light taste”.

A series of aroma defects are caused by enzymatic reactions. These include:

- An unclean taste due to an increased concentration of dimethylsulfide produced by psychotropic microorganisms.
- A fruity taste due to the formation of ethyl esters produced by psychotropic microorganisms, e. g., *Pseudomonas fragii.*
- A malty taste due to increased formation of 3-methylbutanal, 2-methylbutanal, and methylpropanal by *Strept. lactis var. maltigenes.*
- A metallic taste in buttermilk due to (E,Z)-2,6-nonadienol in concentrations >1.3 µg/l. The precursor is the triglycerol-bound α-linolenic acid which is oxidized to 9-hydro-peroxy-10,12,15-octadecatrienoic acid by oxygenases from the starter culture. Proton catalysis liberates (E,Z)-2,6-nonadienal which is reduced to the corresponding alcohol by lactic acid bacteria.
- A phenolic taste due to spores of *Bacillus circulans.*
- A rancid taste due to the release of lower fatty acids (C_4–C_{12}) by milk lipases or bacterial lipases.
- A bitter taste can occur due to proteolytic activity, e. g., on storage of UHT milk. The milk proteinase plasmin is inactivated on intensive heating (142 °C, >16 s). However, some bacterial proteinases can still be active even after much longer exposure to heat (142 °C, 6 min).

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